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August 1951

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1. Dennison, A.D.: *Jour. Med. Soc. New Jersey*, 46:139, Mar., 1949.
2. Saslaw, M.S.: *Jour. Florida Med. Assn.*, 34:657, May, 1948.

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Problem of Estimating the Rate of Secretion of Antidiuretic Hormone in Man
HENRY D. LAUSON 135

The significance of the neurohypophyseal antidiuretic hormone in regulation of the rate of urine flow and in the retention of water in various pathologic states is a matter of controversy. In part this controversy is due to a confusion of methods and interpretation. This meticulous study and analysis by Dr. Lauson critically reviews the relevant literature and adds new data which point up the importance of such factors as total solute excretion and glomerular filtration rate.

Studies of the Serum and Urine Constituents in Patients with Cirrhosis of the Liver during Water Tolerance Tests
ELAINE P. RALLI, STEPHEN H. LESLIE, GEORGE H. STUECK, JR. AND BERTRAM LAKEN 157

The importance of the role of renal regulation of salt and water excretion as a factor in ascites formation in cirrhosis of the liver is becoming more and more apparent. The detailed water tolerance tests described in this paper further emphasize this factor which the authors suggest is under the control, at least in part, of antidiuretic hormone.

Role of Shock in the Production of Central Liver Cell Necrosis
MAX ELLENBERG AND KERMIT E. OSSERMAN 170

The causes of central liver cell necrosis (often confused with chronic congestion of the liver) have always been obscure and diagnosis during life has been difficult, particularly since the various liver function tests have not proved very helpful. In this interesting paper a strong case is made for shock of more than twenty-four hours' duration, whatever the cause, as the principal etiology in ordinary clinical experience, with prolonged hepatic anoxia as the immediately operative factor.

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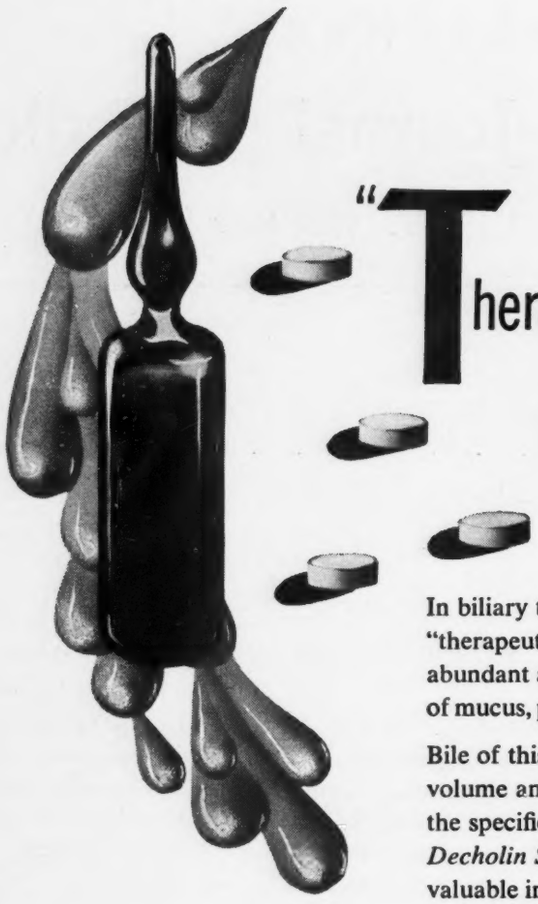

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Antibiotic Therapy of Friedländer Pneumonia
WILLIAM M. M. KIRBY AND DANIEL H. COLEMAN 179

The Friedländer bacillus, K. pneumoniae, is becoming more and more important as a cause of bacterial pneumonia as pneumococcal pneumonias become less frequent and more amenable to antibiotic therapy. This report summarizes eleven cases and describes the results of treatment particularly with chloramphenicol, streptomycin and aureomycin.

Clinical and Pharmacologic Studies with Allylmercaptomethyl Penicillin (Penicillin "O"). . CHARLES V. ADAIR, WILLIAM G. WOODIN AND PAUL A. BUNN 188

Penicillin "O" (allylmercaptomethyl penicillin) was found to have pharmacologic and antibacterial properties analogous to those of penicillin G and is proposed as a substitute for penicillin G when there are untoward reactions.

Allergy to Penicillin O and the Management of Penicillin Sensitivity
SHEPPARD SIEGAL 196

The author describes five cases of skin sensitivity to penicillin O occurring as a result of sensitization with penicillin G, suggesting that penicillin O may not always be a safe substitute for penicillin G in penicillin allergic patients. General comments on the management of penicillin sensitivity conclude the article.

Review

Infectious Mononucleosis. A Study of 210 Sporadic Cases
JOHN E. STEVENS, EDWIN D. BAYRD AND FRANK J. HECK 202

This is a rather superficial but nevertheless instructive analysis of the clinical and laboratory manifestations of infectious mononucleosis as observed in 210 sporadic cases. The findings again emphasize the diverse character and manifold expressions of this common disorder.

Seminars on Arteriosclerosis

Lipid Metabolism and Atherosclerosis. . . . . R. GORDON GOULD 209

Dr. Gould reviews at some length the chemistry of the lipids of clinical interest and their relation to each other and to proteins. He then goes on to consider the biosynthesis of cholesterol and other lipids in animals and man as revealed by isotope tracer methods. This work has already thrown important new light upon problems that are crucial to our slowly increasing knowledge of the pathogenesis of atherosclerosis.

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Diagnostic Criteria of Vascular Headaches

		MIGRAINE	MIGRAINE EQUIVALENTS	MIGRAINE VARIANTS (e.g. Histaminic Cephalgia)	TENSION HEADACHE
BACKGROUND	INCIDENCE	8 to 10 million in U.S.; (ratio of women: men::2.5:1)	As common as migraine	Less common than migraine (mostly in men)	Most common of all headaches
	FAMILIAL HISTORY	Hereditary factor	Hereditary factor	Not significant	Not significant
ONSET OF ATTACK	STAGE OF LIFE	Puberty to menopause	Puberty to menopause	Often after 35	Any
	RATE OF ONSET	Abrupt to gradual	Abrupt to gradual	Abrupt	No pattern
	TIME OF DAY	Usually early morning, diminishing in evening	Variable	Frequent during sleep	Usually at end of day
	DAY OF WEEK, MONTH, ETC.	Most commonly weekends; frequently pre-menstrual and during periods of conflict, tension or stress.	Same as classical migraine	Occur in series, as often as 10 to 15 times a day, often seasonal.	Variable, but usually preceding or following emotional stress or conflict
DESCRIPTIVE QUALITIES OF HEADACHE	DURATION	Minutes to days	Minutes to days	Under 1 hour	No pattern
	NATURE OF HEAD PAIN	Throbbing or pulsating		Constant, boring	Sustained dull or sharp pain
	SITE	Anywhere in head and face—most commonly at right temple, usually unilateral	*SEE NOTE BELOW	Involves eye, neck and often face; unilateral	Most intense in neck, shoulders and occiput—may spread to frontal region—unilateral or bilateral
	TENDERNESS (RESIDUAL)	Near large extra-cranial arteries. Affected region may involve nasal, paranasal, teeth, ear, neck.	Varies according to location	No residual tenderness	Any of head and neck muscles
	EFFECT OF MANUAL PRESSURE	Pressure upon temporal, frontal, post-auricular arteries often reduces intensity	*NOTE Many cases in this classification suffer pain in regions other than the head	Similar to migraine (classical)	Pressure upon tender muscles or regions of tenderness intensifies headache
	EFFECT OF POSITION OF HEAD	Erect position relieves—shaking head aggravates		Pain eased by sitting up and leaning forward	Shaking head reduces intensity by extending muscles
ASSOCIATED SYMPTOMS OF ATTACK	VISUAL DISTURBANCES	Scintillating scotomata; unilateral homonymous hemianopia usually of short duration preceding attack. Photophobia common	Same as classical migraine Photophobia always present in ophthalmic migraine	Photophobia often present	Partial closure of eyes due to muscle spasm. May give impression of faulty vision; Photophobia common
	OTHER OCULAR SYMPTOMS	Injection of conjunctiva and sclera, lacrimation and ptosis of eyelid may occur	Ptosis of eyelid occurs with ophthalmoplegic migraine	Conjunctival injection, lacrimation, nasal stuffiness, discharge	Injection of sclera and conjunctiva; lacrimation may also occur
	VERTIGO AND OTHER SENSORY DISTURBANCES	May be present; paresthesias of hands and face may precede	Paresthesias of hands and face may precede	Infrequent	Infrequent
	MOOD CHANGES	Depression-irritability precede; exaltation follows	Same as classical migraine	No significant changes	Tension, irritability; desire for attention
	GASTRO-INTESTINAL DISTURBANCES	Anorexia, nausea and vomiting at height of attack; flatulence and distension	In gastro-intestinal migraine - flatulence, distension, constipation	Infrequent	Flatulence and distension common especially with severe headache
	CHANGE IN BODY TEMPERATURE	Slight; sometimes unilateral sweating	In some cases up to 104°F.	Not significant	Chills and hot flashes without change in temperature
	TREATMENT	Emotional guidance, general improvement in health; Endocrine therapy. Specific for attack: Cafergot	Emotional guidance, general improvement in health; Specific for attack: Cafergot; vaso-constrictor agent		Sedatives

The information presented in this table has been compiled by the Sandoz Scientific and Research Staffs from the following publications:

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Thyrotoxicosis Factitia. Measurement of the Thyroid Gland I^{131} Uptake and Serum Protein-bound I^{131}

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The author summarizes the scanty information about arachnodactyly, an interesting anomaly, and adds another case.

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This is an interesting case typical of its kind.

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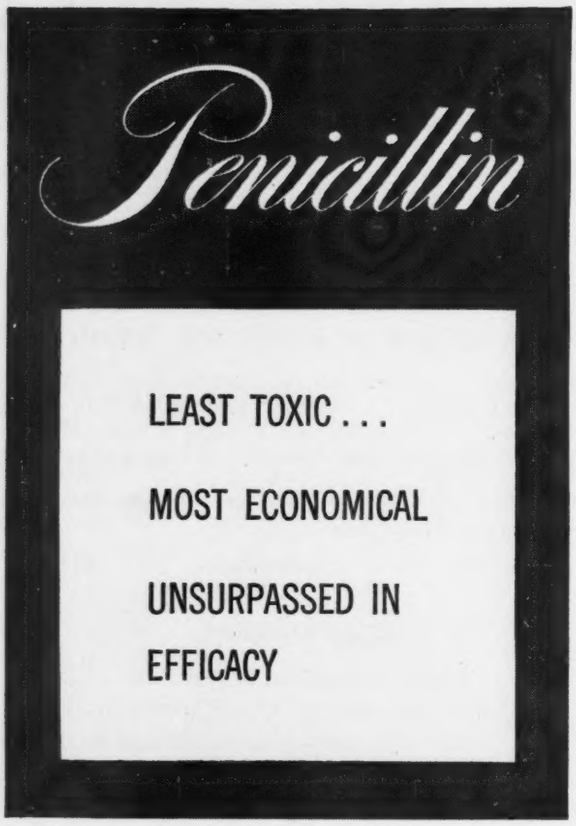

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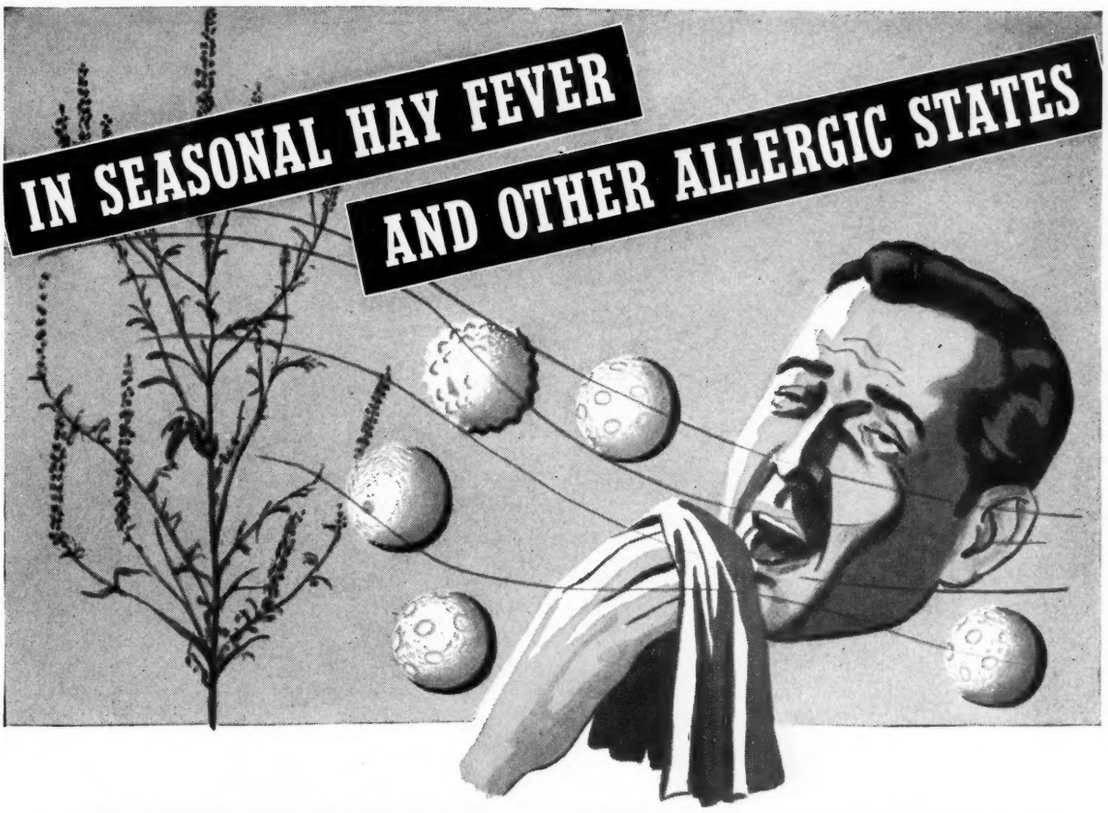

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1. Jeans, P. C.: Feeding of Healthy Infants and Children, J.A.M.A. *142*:806 (Mar. 18) 1950.

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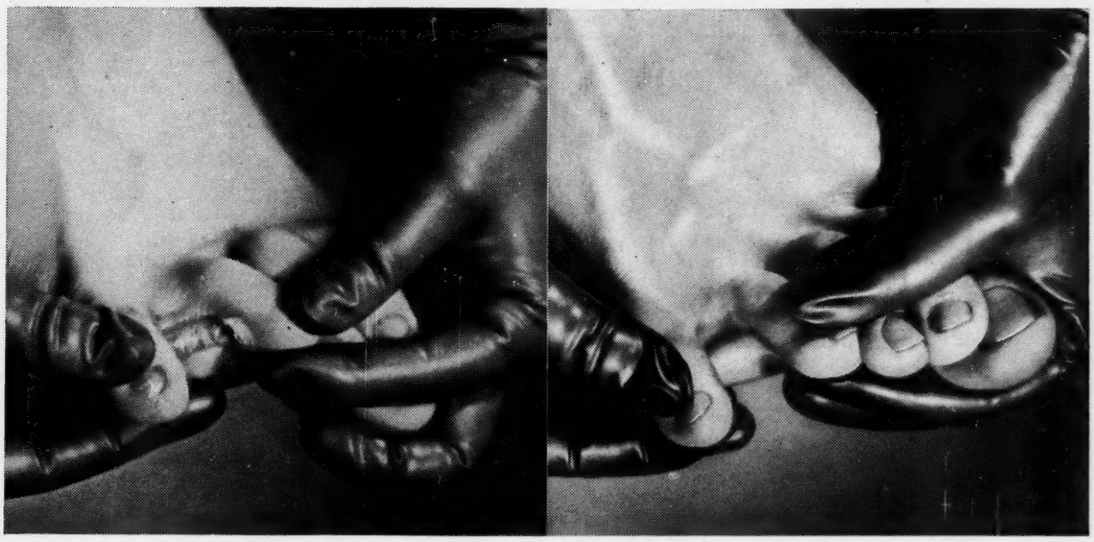

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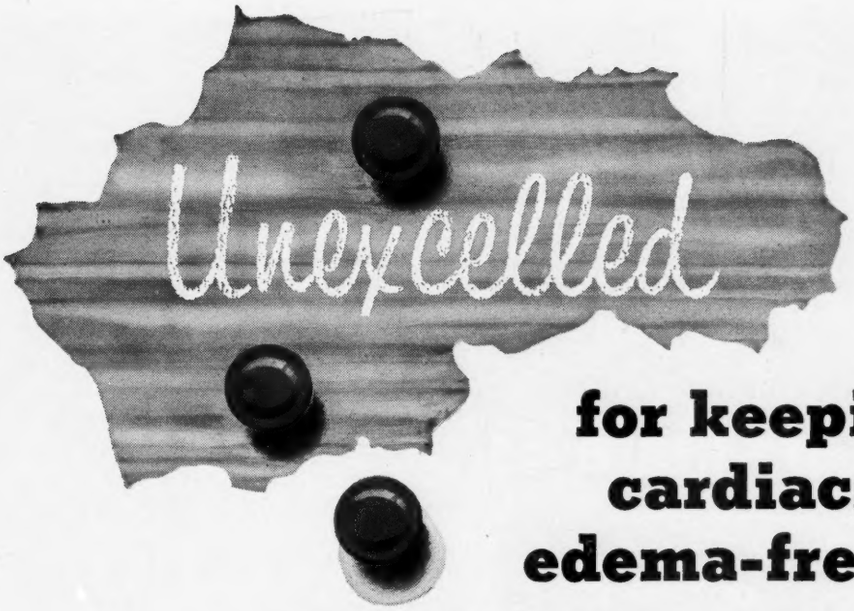

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*Overman, W. J.; Gordon, W. H., and Burch, G. E.: Tracer Studies of the Urinary Excretion of Radioactive Mercury following Administration of a Mercurial Diuretic, Circulation *1*:496, 1950.

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1. Perloff, Wm. H., Treatment of the Menopause. II. In press.

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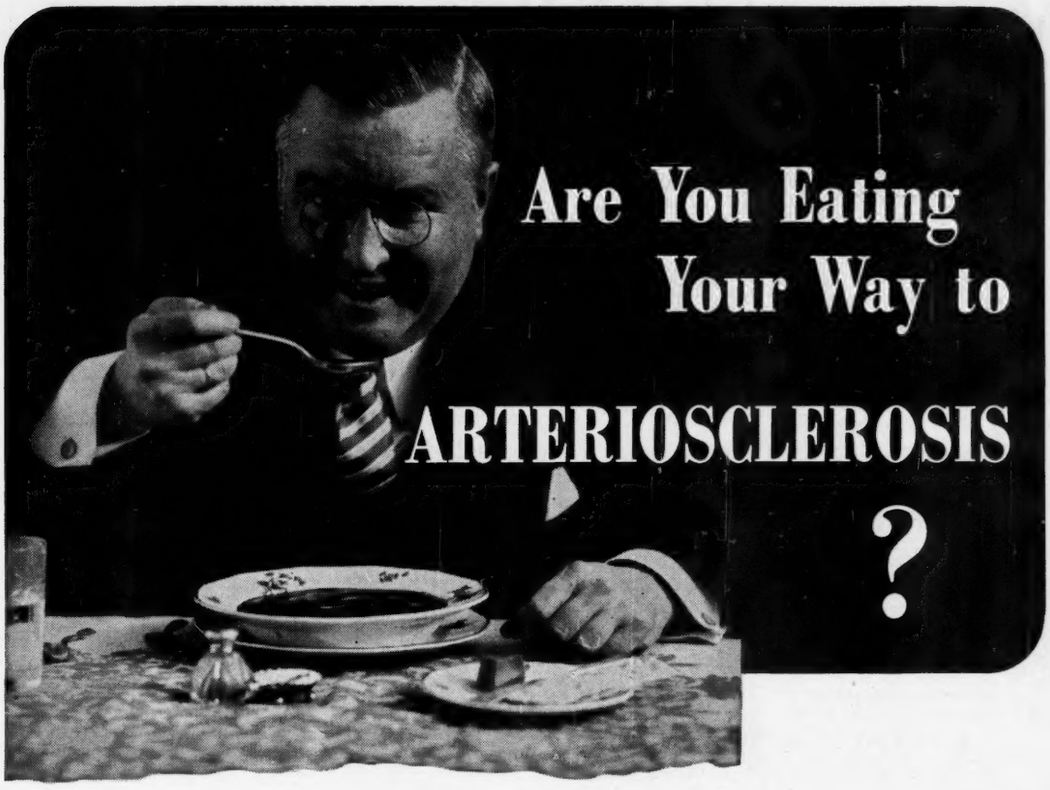

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1. Cannon, P. R.: The Importance of Proteins in Resistance to Infection, J. A. M. A. *128*:360 (June 2) 1945.

2. Strumia, M. M., and Boerner, F.: Phagocytic Activity of Circulating Cells in the Various Types of Leukemia, Am. J. Path. *13*:335 (May) 1937.

3. Mills, C. A., and Cottingham, E.: Phagocytic Activity as Affected by Protein Intake in Heat and Cold, J. Immunol. *47*:503 (Dec.) 1943.

4. Elman, R., and Cannon, P. R.: Protein Malnutrition, in Jolliffe, N.; Tisdall, F. F., and Cannon P. R.: Clinical Nutrition, New York, Paul B. Hoeber, Inc., 1950, chap. 7, p. 192.

5. McLester, J. S.: Nutrition and Diet in Health and Disease, ed. 5, Philadelphia, W. B. Saunders Company, 1949, p. 347.

6. Cannon, P. R.: Recent Advances in Nutrition with Particular Reference to Protein Metabolism, Lawrence, Kansas, Univ. of Kansas Press, 1950, p. 19.

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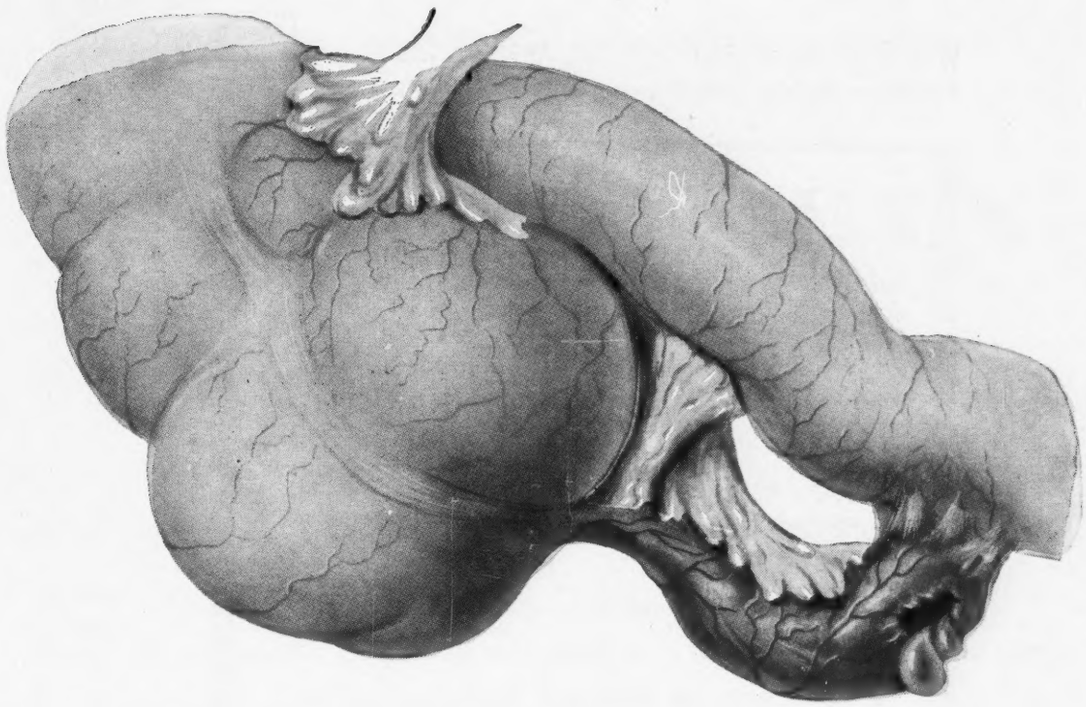

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Schaefjer, J. R., and Pulaski, E. J.:
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1. Heinberg, C.J.: Eye, Ear, Nose & Throat Monthly 30:31 (Jan.) 1951.

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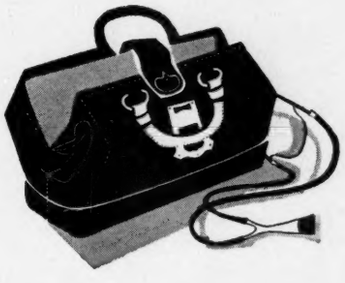

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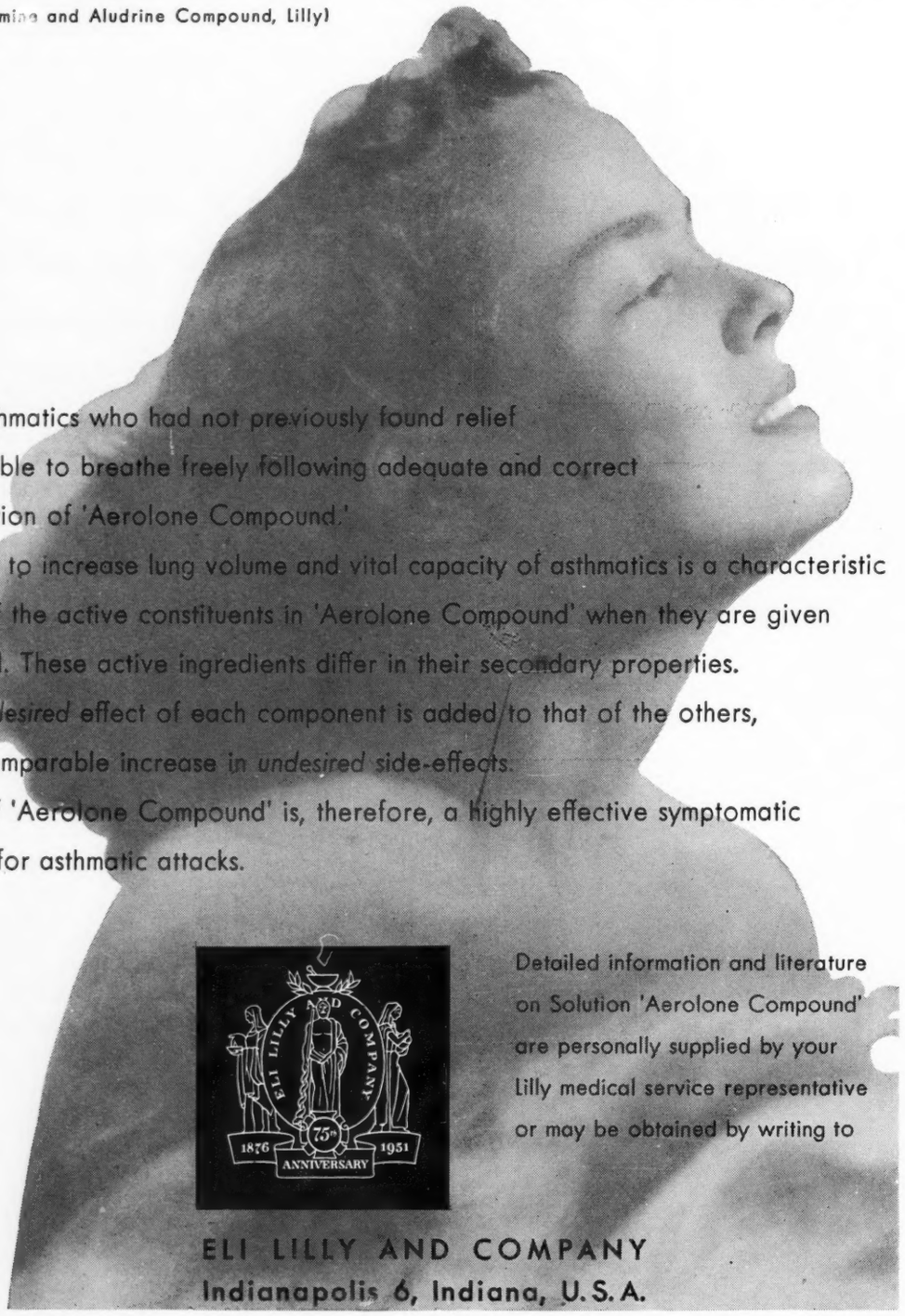

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VOL. XI AUGUST, 1951 NO. 2

Editorial

The Horns of a Dilemma

THE following question, recently put, "Would a person who ate nothing at all live longer than if he ate a salt-free diet containing foods which would result in the excretion of more salt than would occur during fasting?", illustrates the extreme lengths to which preoccupation with a current and fashionable procedure, particularly in the field of treatment, may lead.

Although such an inquiry is highly theoretical and of little practical meaning, there are other aspects of the current use of "sodium-free" diets which are of distinctly important practical significance. Recently, attention has been called to the occurrence of sodium deficiency in individuals maintained on a salt-free diet, with and without other procedures and treatments tending to result in a deficiency of sodium. Excessive intake of fluid, overenthusiastic use of mercurial diuretics, use of solutions of dextrose without accompanying salt may result in further abnormal losses of salt. The symptoms resulting from such deficiency, and serving to call attention to the development of such a complication, are drowsiness, weakness and lethargy, nausea and, occasionally, vomiting, muscular cramps and anorexia. With these symptoms there is found, if there is sufficient awareness of the possibility of the condition to lead to a search for them, a depression of the output of urine, reduction of urinary chlorides to traces or very small amounts, progressive increase in body weight, increase in the nonprotein nitrogen of the blood and decrease in the plasma sodium and chlorides. Some degree of renal insufficiency, either the result of "primary" renal disease or accompanying congestive failure, is a common accompaniment but need not be present. In some cases acidosis resulting from a deficiency of total base (sodium) occurs. This development is, of course, much more likely if renal disease, interfering with ammonia formation by the kidney, is present. Although prompt recognition and proper treatment may result in recovery (which is sometimes spontaneous), renal shut-down is very apt to continue and deaths are believed to be frequent.

Recent experience with two cases exhibiting such a deficiency of sodium has served to emphasize its importance and stress the danger of overenthusiasm with procedures which are effective and currently popular but possess a potential for harm which may not be fully recognized. Both patients died. In one instance there was the complication of the administration over a considerable period of time of a mercurial diuretic which probably led to an increased loss of salt and salt depletion. In the other mercurial diuresis was employed but to a minimal extent and the loss of sodium, as compared with the diminished intake and dilution by parenteral fluids, was likewise minimal.

In the discussion centering around the clinical course and eventual demise of these two patients, the interesting thing other than the details of the symptomatology and findings of disturbed physiology was the

surprise and disappointment expressed by some that a procedure which was found effective and helpful had limitations and that there was a point beyond which it could not be used without as much or more damage to the patient than would result from the spontaneous progress of the disease; that a dilemma was reached in the treatment of congestive failure in which either the disease itself or the untoward effects of the treatment must be endured. Such an attitude indicates too great preoccupation with the admittedly beneficial effects of a procedure and lack of appreciation of the factors limiting its usefulness. The symptoms of salt deficiency have long been known and could be anticipated as the result of a low-sodium diet alone. The influence of other factors in exaggerating the loss or precipitating the symptoms should be clearly apparent. Failure of a mercurial diuretic to cause diuresis should arouse suspicion.

Such dilemmas are, of course, common in medicine. A good example can be drawn from the same field of congestive heart failure. Digitalis is a great benefit in the treatment of congestive failure, with and without the arrythmias in which its use is indicated. There comes a time, however, in the course of nearly every such case when digitalis, despite increasing dosage, is no longer sufficiently effective and any attempt to increase it will do more harm than the disease itself. When that time arrives, the hope of help from digitalis must, however regretful, be given up.

Fortunately, perhaps, the not infrequent failure of the patient to adhere strictly and completely to the difficult and unpleasant extremely low sodium diet has provided a protection that the enthusiasm of the therapist has neglected.

JOHN B. YOUMANS, M.D.

Clinical Studies

The Problem of Estimating the Rate of Secretion of Antidiuretic Hormone in Man*

HENRY D. LAUSON, M.D.
New York, New York

EVIDENCE brought forth during the past two decades suggests that hypersecretion of, decreased destruction of, or increased renal sensitivity to the neurohypophyseal antidiuretic hormone (ADH) may be concerned in the pathogenesis of edema in various diseases.[1–12] These developments have emphasized the need for a better understanding of the role of ADH in relation to other factors influencing the rate of urine flow. The present analysis of new data and critical review of certain important experiments in the literature represent a contribution toward this end.

The rate of liberation of ADH in normal animals and man can be roughly estimated indirectly by determining the dose of posterior pituitary extract required to induce antidiuresis during the course of a maximum water diuresis. Motzfeldt[13] showed in 1917 that very small amounts of such extracts produce antidiuresis in hydrated rabbits. In 1934 Theobald[14] found that about 5 to 10 milliunits (0.005 to 0.01 oxytocic units; see Goodman and Gilman[15] for details of standardization) given intravenously in a single dose reduced the urine flow in man from the peak value following ingestion of 1 L. of water to the control rate. This "minimal inhibition" persisted a few minutes after which the urine flow progressively increased and returned to diuretic levels within an hour. He estimated that in ordinary antidiuresis the antidiuretic hormone (ADH) concentration in the peripheral blood is equivalent to no more than about 0.2 milliunits (mU) of posterior pituitary extract per 100 ml. of blood and that this amount must disappear before water diuresis can occur normally.

Shannon[16] showed that constant intravenous infusion of pituitrin at rates of from 1 to 5 mU per hour restored urine flow (V) in hydrated dogs with experimental diabetes insipidus to rates ordinarily observed in normal dogs. Larger doses not only failed further to reduce V but, because of the enhanced excretion of NaCl, V usually increased slightly. He concluded that the normal rate of liberation of ADH in 10 to 15 kg. dogs varies in the range equivalent to the infusion of about 1 to 5 mU of pituitrin per hour.

The ingenious dog experiments, summarized in Verney's Croonian lecture,[17] led to the conclusion that the rate of ADH liberation induced by a sustained 1 per cent rise in the total osmotic pressure of arterial blood was of the order of 1 μU per second, or 3.6 mU per hour. This rate reduced V to about 10 per cent of the maximum occurring during water diuresis. This estimate of the average rate of liberation accords well with the results of Shannon.[16]

However, Hare, Hare and Phillips,[18] and Chambers, Melville, Hare and Hare,[19] utilizing dogs with experimental diabetes insipidus, demonstrated that in normal dogs the rate of liberation of ADH in response to rapid intravenous infusions of hypertonic NaCl must be much higher than that occurring under ordinary conditions. The latter group concluded from experiments in which pituitrin was added to the saline infusion that dogs with diabetes insipidus required administration of more than 720 mU per hour to restore urine flow and urinary chloride concentration approximately to values observed in normal dogs receiving the hypertonic NaCl only. Essentially the same results were obtained in a parallel study of human diabetes insipidus by Hickey and Hare.[20]

It is evident from these observations that the rate of liberation of ADH varies greatly depending on the degree of plasma hyperosmolarity. It is also apparent from the experiments of Hare and associates[18,19] that the relationship between

* From the Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.

blood ADH concentration and the resulting urine flow is markedly influenced by the rate at which Na and Cl are excreted.

Important contributions to a better understanding of the latter relationship have been made by McCance[21] and Hervey, McCance and Tayler[22] who showed that during hydropenia the increasing excretion of total urinary solutes resulting from administration of hypertonic NaCl or sucrose was associated with a decreasing concentration of urinary solutes. This observation was recently confirmed and greatly extended by Rapoport et al.[23–26] They showed that in osmotic diuresis induced during hydropenia by infusion of a variety of solutes the urinary osmolar concentration decreased uniformly as the total solute excretion increased, regardless of the nature of the loading solute. Thus urine flow in these circumstances was determined by the rate of excretion of unreabsorbed solutes.

It will be assumed, as was possibly implied by these authors,[24] that under the conditions of the experiments just mentioned the antidiuretic hormone is liberated at relatively high rates and that the renal tubules are influenced by ADH to the maximum possible extent. Therefore the equation (Equation 5) derived by these authors[24] relating urine flow to total solute excretion rate may be thought of as describing the changes in urine flow occurring under the condition of maximal ADH action. That is, for any given rate of solute excretion it is assumed that urine flow is the least possible, and that additional ADH would not further diminish the flow. Somewhat similar assumptions regarding these data seem to be implied in recent reviews by Smith[27] and by Pitts and Sartorius.[7] Direct support for the assumption is found in the experiment of Chambers, Melville, Hare and Hare[19] in which administration of 250 mU of pituitrin per hour did not alter the pattern of urine flow in a normal dog during Na_2SO_4 osmotic diuresis.

The experiments to be described in the present report were originally intended to duplicate in man the essential features of Shannon's experiments[16] in order to determine the approximate range of ADH liberation under ordinary conditions. Pitressin was infused intravenously at graduated rates during a state of "physiologic diabetes insipidus"[17] induced by drinking a large volume of water and sustained by drinking water in volumes equal to the urine output. The urine flow and the endogenous "creatinine" urine:plasma concentration ratio (U/P), observed when the infusion had run long enough for the blood pitressin concentration presumably to have approached equilibrium, were taken to be the responses at equilibrium to the particular rate of infusion for the existing rate of total solute excretion. Some of these experiments have been reported previously in preliminary form.[28]

However, certain inconsistencies appeared which may now be explained more adequately on the basis of the studies by Rapoport et al.[23,24] and Hare and associates.[18,19] The data are being reported at this time for the purpose of indicating more precisely the problems involved in estimating the rate of ADH liberation as well as for presenting several such estimates. In addition, observations were made concerning the question of chloruretic activity of posterior pituitary extracts.

The following definitions, assumptions and principles will be used to facilitate the description of the data and the discussion:

1. The term "maximum antidiuresis" is used to imply that the urine flow is the least and the endogenous "creatinine" (or mannitol) U/P the greatest possible for the given rate of total solute excretion (U_sV). Increasing the blood concentration of ADH would not by itself produce further change in V or U/P.

2. It is assumed that for a given rate of total solute excretion (glomerular filtration rate (GFR) held constant) there is a minimum blood ADH concentration which will induce maximum antidiuresis. Higher concentrations will be designated "supraminimal."

3. Since the rate at which the blood is cleared of ADH by inactivation and renal excretion is very rapid,[29–31] the blood concentration of pitressin must begin to fall immediately after a pitressin infusion is discontinued. The extent to which a given blood concentration resulting from a constant infusion of pitressin exceeds the minimum required for maximum antidiuresis must therefore be some direct function of the time during which maximum antidiuresis persists after stopping the infusion.

4. Since the renal response to ADH exhibits a latency not much greater than the kidney-to-bladder delay time,[14,17] the moment at which the blood ADH concentration falls below the minimum required for maximum antidiuresis would be indicated promptly by a rise in V and fall in U/P (assuming that GFR and total solute excretion remain constant). Thus a supramini-

mal pitressin concentration will sustain maximum antidiuresis for some time after stopping the infusion; but when an exactly minimal or a subminimal concentration results from a pitressin infusion, discontinuing the infusion will be followed promptly by a rise in V.

METHODS

A normal male, thirty-four to thirty-seven years of age, weighing about 70 kg. and having a surface area of about 1.84 sq. m., was the subject for the six experiments to be described. In the first four experiments 1,500 ml. of water were ingested during a thirty- to forty-minute period after a light breakfast of two or three slices of bread and jam. He then travelled from his home to the hospital which included a walk of four blocks. Immediately after each voiding 5 per cent glucose in water was drunk in a volume equal to the urine output. The glucose was included to prevent possible neurogenic disturbance of the neurohypophysis due to hunger since no food was taken during the experiment. An intravenous infusion of physiologic saline was begun and continued at a rate of about 1 ml. per minute. This rate was chosen because it approximately replaced the NaCl excreted in the urine and roughly balanced the insensible water loss. The subject remained recumbent and sweating did not occur, except as noted. He remained relaxed despite the invariable development of a fronto-occipital headache and aching in the back of the neck, the intensity of which increased progressively with time.

After water diuresis had become maximal a solution of commercial pitressin,* freshly diluted with physiologic saline, replaced the control infusion. In Experiment 1, performed in September, 1947, the initial rate was 7.5 mU per hour. After two hours the urine flow had stabilized and a second dilution of pitressin in saline replaced the first. Two hours later a third rate of pitressin infusion was begun. The volume of solution infused per minute remained approximately constant throughout. Urine flow again reached equilibrium within two hours. At this time the infusion was stopped and the subject got up and walked around leisurely; slight perspiration was noted for the first time.

In Experiment 2, two weeks later, only a high rate of pitressin administration was studied (295 mU per hour). In Experiment 3, conducted in February, 1948, five months after the first two studies, the initial rate was 38 mU per hour; after two hours this was reduced to 16 mU per hour.

Experiment 4 was carried out after a lapse of over two years on May 6, 1950. This time the subject spent the night in the hospital in order that control urine could be collected after 6 A.M. during recumbency and without antecedent physical activity. No fluid was taken after 9 P.M. the night before.

A fifth and sixth experiment will be described in which a constant infusion of mannitol was administered throughout the test; the clearance of mannitol was used to approximate the glomerular filtration rate. These experiments were actually conducted in November, 1946, as preliminary trials of the effects on chloride excretion of the oxytocic substance, pitocin, as well as of pitressin. The procedure differed from that in Experiments 1 to 4 in certain other respects which will be described with the results.

The subject voided without catheterization, usually in the standing position. Collection periods ranged from ten to sixty-five minutes. Blood for plasma creatinine and chloride analysis was drawn at intervals of one and one-half to two and one-half hours.

Total endogenous creatinine-like chromogen was determined in 1:5 tungstate filtrates of plasma (Wu method[32]) and in diluted urines by the Phillips modification[33] of the Jaffé reaction. Mannitol was measured by a slight modification of the method of Corcoran and Page[34] using cadmium sulfate for plasma protein precipitation (Goldring and Chasis[35]). Plasma and urine chlorides were determined by the Van Slyke and Hiller modification[36] of Sendroy's silver iodate method.[37] Sodium and potassium were measured

* Ampoules from a single lot F 743 M, purchased late in October, 1946, and stored at room temperature thereafter, were used in all of the experiments. The original manufacturer's assay, conducted October 10, 1946, showed 20 pressor units per ml. Through the courtesy of Dr. A. C. Bratton, Jr., of Parke, Davis and Co., 2 ampoules were re-assayed early in June, 1950, and both showed 10 pressor units per ml., a deterioration of 50 per cent, due undoubtedly to the relatively high storage temperature. If as a rough approximation the rate of deterioration is assumed to be proportional to the residual activity, the estimated strength, relative to the original labelled potency, would be as follows: Exp. 1, 84 per cent; Exp. 2, 83 per cent; Exp. 3, 77 per cent; Exp. 4, 51 per cent; Exps. 5 and 6, 98 per cent. In general, these quantitative discrepancies do not significantly affect the interpretations made from the data. In the legend to each Figure the estimated residual activity will be noted.

in a Perkin-Elmer Model 52A flame photometer with lithium as internal standard. Urea was determined by the hypobromite method of Van Slyke and Kugel.[38]

The clearances of endogenous "creatinine" and mannitol (C_{cr}, C_m) were calculated in the

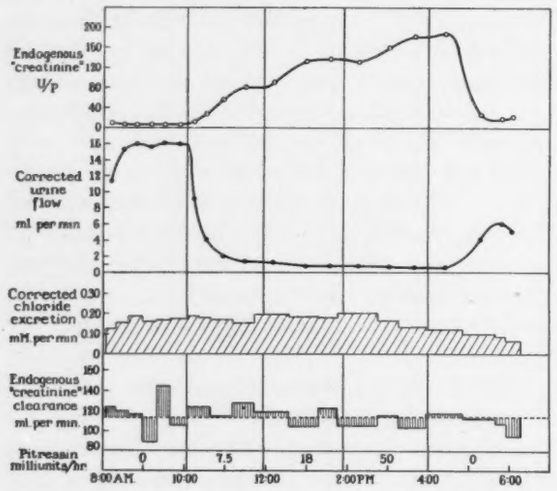

FIG. 1. Data of Exp. 1, September 11, 1947. Urine flow and chloride excretion are "corrected" as described in text, the factor for each period being inversely proportional to the deviation of the "creatinine" clearance from the mean value indicated by the interrupted line. At the time of this study the estimated residual activity of pitressin was 84 per cent of that indicated in the graph, or about 6.3, 15.1 and 42 mU per hour, respectively.

usual manner. As an approximation, changes in the U/P ratio of "creatinine" or mannitol were assumed to be interchangeable estimates of changes in tubular reabsorption of water. In this subject the mannitol clearance measured in fifty-five periods in Experiments 5 and 6 and on other occasions averaged 118 ml. per minute with a standard deviation of ± 9 ml. per minute, whereas the "creatinine" clearance in the eighty periods of Experiments 1 to 4 averaged 121 ± 12 ml. per minute. Since these clearances with few exceptions varied relatively little during each experiment, except in a manner suggesting incomplete bladder emptying, the urine flow and solute excretion values shown in the Figures have been "corrected" as follows:

$$\text{"corrected" V (and } U_xV) = \frac{\text{mean } C_{cr} \text{ (or } C_m)}{\text{observed } C_{cr} \text{ (or } C_m)} \times \text{observed V (and } U_xV)^*$$

* Because of variation in the duration of the periods this assumes a constant clearance throughout each experiment and cancels bladder emptying errors, which in a few periods appear to have been fairly large. The rates of urine flow and solute excretion shown in the Tables are the uncorrected observed values.

RESULTS AND COMMENTS

Response of Urine Flow and "Creatinine" U/P to Graded Rates of Pitressin Administration. Figure 1 and Table I show the effects of administering pitressin at successive rates of 7.5, 18 and 50 mU per hour in Experiment 1. Stabilization of the "creatinine" U/P by the end of each infusion period indicates that the pitressin concentration in the blood had practically attained equilibrium by that time. It is assumed that at these times the rate of pitressin infusion equalled the rate of its removal from the blood by inactivation, diffusion into tissues and urinary excretion.[29] The equilibrium values of U/P were 7.2, 81, 138 and 183 for the infusion rates of 0, 7.5, 18 and 50 mU per hour, respectively. It is to be noted that U/P did not fall immediately after stopping the final infusion. The significance of this fact will be discussed later. The return of water diuresis (peak urine flow occurring about one and one-half hours after the end of the infusion) is presumptive evidence that the endogenous secretion of antidiuretic hormone had been almost completely inhibited during the entire nine hours of the experiment. It is concluded, therefore, that practically all of the increase in U/P was due to the exogenous extract. The excretion of chloride was fairly constant until the middle of the 50 mU per hour pitressin infusion after which it progressively decreased. At the end of this infusion the subject got up and walked around leisurely. In association with the change of posture and activity the chloride excretion continued to decrease.

Figure 2 and Table II present the results of Experiment 2 in which pitressin was administered at the relatively high rate of 295 mU per hour. It is interesting that the "creatinine" U/P at equilibrium was only 134 and that a sub-

the integrated mean creatinine and mannitol clearances for each experiment were calculated as:

$$\text{Mean clearance} = \frac{\Sigma(C_1D_1 + C_2D_2 + \cdots + C_nD_n)}{\Sigma(D_1 + D_2 + \cdots + D_n)},$$

where C and D refer to the clearance in ml. per minute and duration in minutes of the individual periods, respectively.

stantial increase followed cessation of the infusion. The return of diuresis began considerably later than in the first experiment, as would be expected from the fact that the infusion rate was much higher.

In Figure 3 and Table III from Experiment 3 it is seen that 38 mU per hour increased the U/P to 194, whereas in the following periods a rate less than half as great induced a slight additional increase to 209. That as little as 16 mU per hour should produce a higher U/P ratio than was observed in Experiment 1 with 50 and in Experiment 2 with 295 mU per hour may seem puzzling at first. The prompt return of the diuresis after stopping the infusion of 16 mU per hour in Experiment 3 indicates that the infusions had not been accidentally switched and furthermore that no large decrease in the blood clearance of pitressin had occurred between Experiment 1 and Experiment 3. It is possible that the pitressin used in this experiment was more potent but since the ampoule was from the same lot as

TABLE I*

EXPERIMENT 1, SEPTEMBER 11, 1947

Period	Time	Cumulative Balance		Urine Flow (ml./min.)	Endogenous "Creatinine"		Plasma Chloride Concentration (mM/L.)	Urinary Chloride	
		Water (ml.)	Chloride (mM)		U/P	Clearance (ml./min.)		Concentration (mM/L.)	Excretion (mM/min.)
	8:00 A.M.	Begin infusion I—saline control							
1	8:04– 8:20	1,459	2.6	12.31	10.1	124	106.9	11.1	0.137
2	– 8:39	1,459	1.6	16.00	7.5	120		11.2	0.168
3	– 8:59	1,460	0.4	16.30	7.2	117		11.8	0.192
4	– 9:20	1,460	0.5	12.15	7.3	89		10.4	0.126
5	– 9:40	1,461	—1.3	20.35	7.1	145		10.6	0.216
6	9:40–10:06	1,469	−1.2	14.73	7.2	106		10.9	0.161
	10:02	Begin infusion II—7.5 mU of pitressin per hour							
7	–10:23	1,477	−1.4	9.89	12.6	124		21.0	0.208
8	–10:40	1,484	−1.5	4.41	28.2	124		44.7	0.197
9	–11:14	1,498	−0.9	2.06	55.9	115		84.5	0.174
10	–11:45	1,514	−0.3	1.58	80.8	128	106.5	109.4	0.173
	11:54	End infusion II							
	11:58	Begin infusion III—18 mU of pitressin per hour							
11	11:45–12:36	1,519	−3.8	1.30	91.5	119		157.6	0.206
12	– 1:20	1,524	−5.0	0.78	133	105		215.4	0.169
13	– 1:48	1,527	−6.2	0.89	138	123	107.7	220.0	0.196
	1:54	End infusion III							
	1:56	Begin infusion IV—50 mU of pitressin per hour							
14	1:48– 2:46	1,525	−9.5	0.79	132	105		234.2	0.186
15	– 3:18	1,527	−10.8	0.72	160	115		233.0	0.167
16	– 3:59	1,528	−10.6	0.56	183	103	107.5	221.3	0.124
	4:00	End infusion IV							
17	3:59– 4:52	1,453	−17.3	0.62	188	117		203.2	0.129
18	– 5:42	1,211	−21.3	4.04	28.0	113		24.4	0.099
19	– 5:57	1,110	−22.6	5.68	18.8	107		14.5	0.082
20	– 6:13	1,046	−23.4	3.97	23.4	93		13.8	0.055
				Integrated mean value 114					

* Between 6:55 and 7:40 A.M. 1,500 ml. of water and two slices of dry bread were ingested. Balances were considered to be zero at 7:00 A.M. Water balance is the volume of fluid given orally and intravenously minus the urine volume and a constant quantity, 0.8 ml. per minute, representing assumed insensible water loss.[39] Chloride balance is calculated as the difference between intravenous intake and urinary excretion. The cumulative balances are calculated to the end of each period. From 7:00 A.M. through period 15, water containing 5 per cent glucose was drunk immediately after each voiding in a volume equal to the urine output. The average rates of infusion were: I, 0.83 ml. per minute; II, 1.25 ml. per minute; III, 0.91 ml. per minute; IV, 0.83 ml. per minute. The subject was recumbent, except for voiding, through period 16; thereafter, he was either ambulatory or sitting and for the first time noted a small amount of perspiration.

those used previously this seems unlikely. It is also possible that a change in the kidney sensitivity had occurred in the five-month interval between Experiments 1 and 3. The most likely reason, however, is that the rate of total solute excretion in Experiment 3 was probably less than in Experiment 1, as is suggested by the lower rate of chloride excretion. The relationship of antidiuresis to the concurrent solute excretion will be discussed below. Somewhat similar variation in the antidiuretic response in the same dog on different occasions is seen in the data of Shannon.[16]

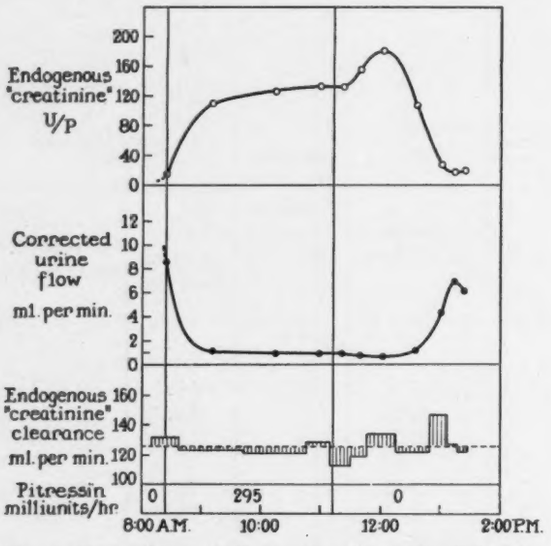

FIG. 2. Data of Exp. 2, September 29, 1947. Chloride excretion was not measured. Urine flow is "corrected" as in Figure 1. At this time the estimated residual activity of pitressin was 83 per cent of that indicated in the graph, or about 245 mU per hour.

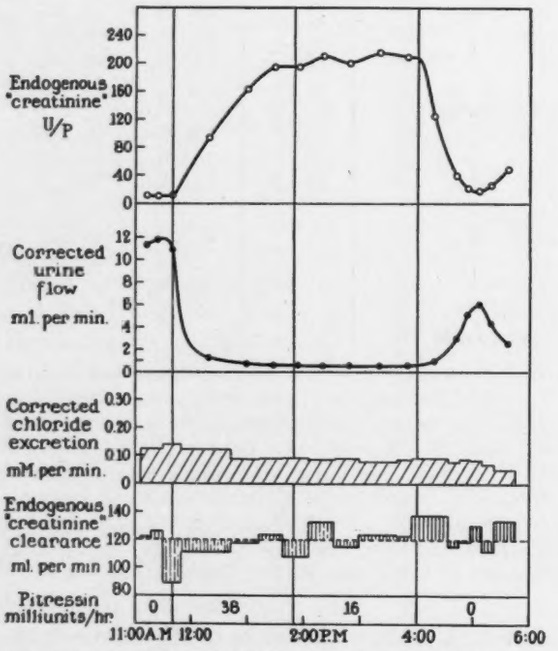

FIG. 3. Data of Exp. 3, February 21, 1948. Urine flow and chloride excretion are "corrected" as in Figure 1. At this time the estimated residual activity of pitressin was 77 per cent of that indicated in the graph, or about 29.2 and 12.3 mU per hour, respectively.

TABLE II*
EXPERIMENT 2, SEPTEMBER 29, 1947

Period	Time	Cumulative Water Balance (ml.)	Urine Flow (ml./min.)	Endogenous "Creatinine" U/P	Endogenous "Creatinine" Clearance (ml./min.)
	A.M.				
0	8:10– 8:38	1,422	9.04	14.6	132
	8:25	Begin infusion—295 mU of pitressin per hour			
1	8:38– 9:43	1,353	1.11	111	123
2	–10:46	1,295	0.95	127	121
3	–11:11	1,272	0.96	134	129
	11:12	End infusion			
4	11:11–11:31	1,239	0.85	133	113
5	–11:48	1,212	0.76	156	119
6	–12:17	1,167	0.74	181	134
7	–12:51	1,102	1.12	109	122
8	– 1:08	999	5.11	28.8	147
9	– 1:18	920	7.05	18.0	127
10	– 1:28	855	6.00	20.3	122
				Integrated mean value	125

* Between 6:40 and 7:15 A.M. 1,500 ml. of water and two slices of bread and jam were ingested. Water balance was considered to be zero at 7:00 A.M. Cumulative balances were calculated as in Table I. From 7:00 A.M. through period 0 water was drunk immediately after voiding in a volume equal to the urine output. The rate of infusion was 0.84 ml. per minute. The subject was recumbent, except for voiding, through period 3; thereafter he was either ambulatory or sitting. No sensible perspiration was noted at any time.

Experiment 4 was primarily an attempt to reproduce the interesting results of Experiment 2, in which a high rate of infusion raised "creatinine" U/P to only 134 at equilibrium, and in which U/P rose after termination of the infusion to 180 before finally falling as water diuresis returned. The procedure differed from the previous studies as follows: two preliminary urine collections were made under strictly basal conditions before drinking 1,500 ml. of water; the subject remained recumbent and the slow

saline infusion was continued almost to the end of the experiment; urinary urea, sodium and potassium were determined in addition to chloride in order to account for most of the solute excretion.

the mean value for the entire experiment as shown in Figure 4 and Table IV. The U/P, which was 206 initially, fell to 6.4 after water ingestion, the lowest ever observed in this subject.* During the infusion of 375 mU of pitressin

TABLE III*
EXPERIMENT 3, FEBRUARY 21, 1948

Period	Time	Cumulative Balance		Urine Flow (ml./min.)	Endogenous "Creatinine"		Plasma Chloride Concentration (mM/L.)	Urinary Chloride	
		Water (ml.)	Chloride (mM)		U/P	Clearance (ml./min.)		Concentration (mM/L.)	Excretion (mM/min.)
	A.M.								
	10:15			Begin infusion I—saline control					
9	11:06–11:18	1,677	−1.2	11.47	10.67	122	105.5†	10.9	0.125
10	–11:30	1,677	−1.2	12.42	10.12	126		10.3	0.128
	11:35			End infusion I					
	11:40			Begin infusion II—38 mU of pitressin per hour					
11	11:30–11:50	1,673	−1.4	8.09	10.98	89		12.8	0.104
12	–12:43	1,676	−0.4	1.18	94.2	111		95.4	0.112
13	– 1:14	1,677	0.9	0.73	163	118	104.7	122	0.089
14	– 1:39	1,662	1.9	0.64	194	124		146	0.093
	1:43			End infusion II					
	1:51			Begin infusion III—16 mU of pitressin per hour					
15	1:39– 2:07	1,674	1.5	0.55	195	108		148	0.082
16	– 2:34	1,659	1.2	0.63	211	133		156	0.098
17	– 3:00	1,678	1.1	0.58	200	115	104.8	145	0.087
18	– 3:40	1,659	1.5	0.57	216	124		140	0.081
19	– 3:57	1,650	1.5	0.59	209	123	104.9	146	0.086
	4:00			End infusion III					
20	3:57– 4:35	1,581	12.5	1.10	125	138		96.2	0.106
21	– 4:46	1,539	11.6	2.87	40.2	115		26.7	0.074
22	– 4:59	1,465	10.5	5.12	23.2	119		16.7	0.086
23	– 5:11	1,476	9.4	6.58	19.8	130		13.6	0.090
24	– 5:24	1,309	8.6	4.11	27.2	112		15.1	0.062
25	– 5:47	1,226	7.3	2.82	47.4	134	104.7	19.5	0.055
				Integrated mean value 120					

* Between 6:40 and 7:35 A.M. 1,500 ml. of water and three slices of bread and jam were ingested. Balances were considered to be zero at 7:00 A.M. Cumulative balances were calculated as in Table I. After 7:00 A.M., through period 17, water containing 5 per cent glucose was drunk immediately after each voiding in a volume equal to the urine output. At 8:18 A.M. a saline control infusion was begun, but it was apparently contaminated with a small amount of pitressin, since the urine flow fell gradually to 1.2 ml. per minute by 9:45 A.M. At 10:10 this infusion was ended and at 10:15 replaced by fresh saline. Because the contaminating dose of pitressin is unknown, these periods are omitted.

The rates of infusion were: I, 0.79 ml. per minute; II, 0.85 ml. per minute; and III, 0.89 ml. per minute. The subject was recumbent, except for voiding, through period 19; thereafter he was either ambulatory or sitting. In period 20 he ate a ham sandwich.

† Blood drawn at 9:15 A.M.

As seen in Table IV the control urine flows of 0.47 and 0.50 ml. per minute were associated with "creatinine" clearances of 98 and 100 ml. per minute, respectively, values which are definitely below those following water ingestion. After completion of the pitressin infusion the clearance tended to be somewhat higher than

per hour the maximum U/P was only 122. Unlike Experiment 2, however, the U/P did not rise after stopping the pitressin infusion but remained fairly stable until period 25, an inter-

* Examination in the several experiments of the minimum "creatinine" U/P values in relation to the concurrent rates of solute excretion gives support to the

val of over one hour; after this it fell rapidly to a minimum of 13.4 at the peak of the subsequent water diuresis. Urine flow varied inversely with U/P and by the end of the pitressin infusion

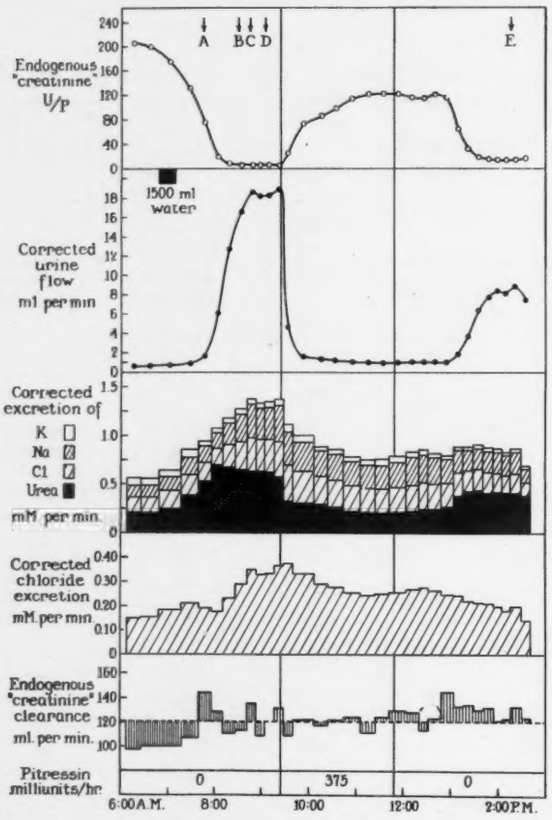

FIG. 4. Data of Exp. 4, May 6, 1950. Urine flow and solute excretions are "corrected" as in Figure 1. At A (7:45 A.M.) the control intravenous infusion of physiologic NaCl was begun at 1 ml. per minute; B (8:30 A.M.), the needle clotted and no saline entered the vein for fifteen minutes, C (8:45 A.M.), the infusion was re-established but inadvertently at a rapid rate; D (9:04 A.M.), it was slowed again to about 1 ml. per minute; E, the after-control saline infusion was terminated. Note that the rates of sodium and chloride excretion had almost reached a maximum value before the rapid saline infusion started at C. At the time of this experiment the estimated residual activity of pitressin was 51 per cent of that indicated in the graph, or about 190 mU per hour.

reached a minimum value of about 1 ml. per minute.

The solute concentrations and excretions are of interest in this experiment and probably explain why this relatively high rate of pitressin infusion failed to decrease the urine flow below 1 ml. per minute. The solute concentration was highest and the solute excretion lowest in periods 1 and 2 in which the urine flow was truly oliguric. During the sustained diuresis in periods 8 to 12 the reverse was true, the solute excretion having risen to nearly 1.5 mM per minute. Under pitressin influence the excretion of each of the individual solutes decreased but their combined rate at the time of equilibrium in periods 18 and 19 was still about 70 per cent above that in period 1. After the pitressin infusion stopped the solute excretions increased again by a small amount. These observations will be discussed more fully subsequently.

Experiment 5, in which mannitol was administered at a constant rate throughout, is summarized in Table v. The plasma concentration ranged from 42 to 47 mg. per 100 ml. (2.3 to 2.6 mM/L.). This experiment also differed from the others in that less water was drunk initially, a lower water balance was maintained and the net loss of chloride was greater. After reaching a steady state of diuresis the oxytocic fraction pitocin was infused at the rate of 30 mU per hour. The very slight antidiuresis observed may well have been due to the small content of pressor (antidiuretic) fraction in this preparation.* There was only the slightest suggestion of chloruretic activity associated with the pitocin infusion. Infusion of 42 mU of pitressin per hour for 1.5 hours during periods 18 to 20 reduced the urine flow to 1.4 ml. per minute and raised the mannitol U/P to 84.9. It is assumed that these values were near to equilibrium for this rate of infusion and furthermore that the blood pitressin concentration achieved was supraminimal. After terminating the pitressin infusion the patient remained recumbent and the diuresis returned to the control level by the last period. Chloride excretion was essentially constant, especially if "corrected" for variations in the concurrent mannitol clearance,

recent observation of Rosenbaum, Nelson and Strauss[40] that maximum urine flow in water diuresis is related to electrolyte excretion. A decreased solute excretion is probably one of the reasons that the maximum urine flow in the post-pitressin diureses of Exps. 1 to 4 was less than that observed at the beginning of the respective studies. The differences among the maximum control urine flows in Exps. 1, 3 and 4 are likewise correlated with the differences in chloride excretion. This phenomenon indicates that even in water diuresis there is an "osmotic" component. In other words, the concurrent total solute (not only electrolyte) excretion exerts an osmotic influence on the urine flow regardless of the blood ADH concentration.

* Dr. Oliver Kamm, of Parke, Davis & Co., advises that commercial pitocin contains no more than 1 unit of pressor substance per 20 units of oxytocic substance.

TABLE IV*
EXPERIMENT 4, MAY 6, 1950

Period	Time	Cumulative Balances		Urine Flow (ml./min.)	Endogenous "Creatinine"		Urea Clearance (ml./min.)	Plasma Chloride Concentration (mM/L.)	Urine Concentration					Urinary Excretion				
		Water (ml.)	Chloride (mM)		U/P	Clearance (ml./min.)			Chloride (mM/L.)	Sodium (mM/L.)	Potassium (mM/L.)	Urea (mM/L.)	Cl + Na + K + Urea (mM/L.)	Chloride (mM/min.)	Sodium (mM/min.)	Potassium (mM/min.)	Urea (mM/min.)	Cl + Na + K + Urea (mM/min.)
	A.M.																	
1	6:07– 6:27	−25	−2.4	0.47	206	98	25.8		254	209	130	359	952	0.121	0.099	0.062	0.170	0.452
2	– 6:49	−54	−5.3	0.50	200	100	25.8		260	220	109	341	930	0.130	0.110	0.054	0.170	0.464
	6:50– 7:10	1,500 ml. of water ingested																
3	6:49– 7:18	1,406	6.3	0.57	175	100	31.0		264	212	98.5	360	934	0.150	0.121	0.056	0.205	0.532
4	– 7:40	1,388	2.1	0.82	131	107	51.7		231	196	92.5	418	937	0.188	0.160	0.076	0.342	0.766
	7:45	Begin infusion I—saline control																
5	7:40– 7:57	1,406	4.1	1.88	76.9	145	96.5	105.2	121	110	29.3	339	599	0.288	0.208	0.055	0.638	1.129
6	– 8:11	1,407	3.6	6.43	20.0	129	112.8		29.6	27.4	6.10	115	178	0.190	0.176	0.039	0.740	1.145
7	– 8:27	1,410	2.6	11.75	9.45	111	96.3		18.2	17.6	3.66	52.7	92.2	0.214	0.207	0.043	0.620	1.084
	8:30– 8:45	Infusion stopped for 15 minutes, then resumed at increased rate																
8	8:27– 8:43	1,419	−1.7	15.63	7.29	114	96.8		17.2	16.9	3.12	39.3	76.5	0.269	0.264	0.049	0.614	1.196
9	– 8:54	1,563	21.3	20.95	6.48	136	114.2		18.6	18.2	2.97	34.1	73.9	0.390	0.381	0.062	0.715	1.548
10	– 9:04	1,715	43.1	16.50	6.64	109	92.3		18.1	17.5	3.14	34.8	73.5	0.299	0.289	0.052	0.574	1.214
11	– 9:17	1,720	40.8	18.30	6.61	121	101.8		18.1	18.0	3.30	34.2	73.6	0.331	0.330	0.060	0.626	1.347
12	– 9:28	1,723	38.1	20.60	6.41	132	104.2		19.2	19.0	3.50	30.7	72.4	0.396	0.391	0.072	0.632	1.491
	9:21	End infusion I																
	9:25	Begin infusion II—375 mU of pitressin per hour																
13	9:28– 9:40	1,725	38.5	4.17	26.0	109	48.2		80.6	74.0	15.1	69.4	239	0.336	0.308	0.063	0.289	0.996
14	–10:08	1,732	36.2	1.67	73.0	122	51.9	106.4	200	184	36.0	183	603	0.335	0.308	0.060	0.306	1.009
15	–10:25	1,737	35.4	1.35	86.9	118	49.8		205	192	31.5	213	641	0.278	0.261	0.043	0.288	0.870
16	–10:45	1,742	34.3	1.25	97.9	122	47.1		224	217	36.2	214	691	0.280	0.272	0.045	0.267	0.864
17	–11:06	1,747	33.4	1.09	114	125	42.2		240	237	42.8	216	736	0.263	0.260	0.047	0.237	0.807
18	–11:25	1,751	33.0	0.92	121	112	36.0		245	239	53.0	214	751	0.226	0.220	0.049	0.198	0.693
19	–11:44	1,755	32.1	1.03	122	125	40.3		249	240	60.8	214	764	0.255	0.247	0.062	0.220	0.784
	11:49	End infusion II																
	11:58	Begin infusion III—saline control																
20	11:44–12:04	1,752	29.8	1.07	121	130	42.8	106.7	255	257	60.3	216	788	0.274	0.277	0.065	0.232	0.848
21	–12:21	1,759	28.0	1.12	116	129	45.2		257	270	52.0	218	797	0.287	0.302	0.058	0.244	0.891
22	–12:33	1,764	27.1	1.00	114	114	43.5		258	267	48.5	232	805	0.258	0.267	0.048	0.232	0.805
23	–12:49	1,769	25.7	1.03	120	124	47.4		260	266	43.3	244	803	0.268	0.275	0.045	0.252	0.840
24	– 1:04	1,775	24.1	1.27	115	146	62.4		232	232	33.8	259	757	0.294	0.294	0.043	0.328	0.959
25	– 1:16	1,780	23.0	2.08	64.5	134	80.0		129	124	21.0	202	476	0.269	0.268	0.044	0.420	1.001
26	– 1:30	1,786	22.2	4.14	32.7	135	91.8		59.3	54.9	10.0	116	240	0.245	0.228	0.041	0.480	0.994
27	– 1:42	1,790	21.6	6.96	18.8	131	92.4		33.4	33.5	5.39	68.7	141	0.232	0.233	0.038	0.478	0.981
28	– 1:55	1,785	21.0	8.48	15.6	133	87.7		27.4	28.5	4.45	53.2	114	0.232	0.242	0.038	0.451	0.963
29	– 2:06	1,703	20.8	8.45	14.4	122	83.3		23.7	26.3	3.75	50.2	104	0.200	0.222	0.032	0.424	0.878
30	– 2:16	1,627	20.9	8.30	14.9	124	83.0		22.3	25.5	3.83	50.9	103	0.185	0.212	0.032	0.423	0.852
	2:16	End infusion III																
31	– 2:30	1,471	17.7	9.93	13.4	134	89.5	104.8	22.4	25.4	3.40	45.6	96.8	0.222	0.252	0.034	0.453	0.961
32	– 2:42	1,370	15.9	7.66	16.3	125	77.2		19.1	18.1	4.51	50.7	92.4	0.146	0.139	0.035	0.388	0.708
		Integrated mean value 121																

* At 7:00 A.M. the subject ate three slices of bread and jam. Balances were considered to be zero at 6:07 A.M. Cumulative balances were calculated as in Table I. Immediately after voiding in periods 1 through 28 water containing 5 per cent glucose was drunk in a volume equal to the urine output. In periods 13 through 20 the drinking solution contained 50 mM per L. of NaCl in addition to glucose. The average rates of infusion I were: approximately 1 ml. per minute during periods 5, 6 and 7; 0 during period 8; inadvertently very rapid (*ca.* 16 ml./min.) during periods 9 and 10; and again 1 ml. per minute during periods 11 and 12. The rates of infusion II and III were 1.04 and 1.19 ml./mn., respectively.

The subject remained recumbent, except for voiding, during all periods except 31 and 32. There was no sensible perspiration. Plasma concentrations of sodium, measured at the same time as plasma chloride, were 141.3, 140.0, 140.0 and 139.8 mM per L., respectively. Since the hypobromite method also measures urinary ammonia nitrogen, expressing the analytic results as mM/L. of urea slightly underestimates the molar value of the sum of urea + ammonia.

at about 0.25 mM per minute until period 20; after this it declined slowly to 0.17 mM per minute by the last period. This fall was associated with a decrease in plasma chloride from 102.8 to 99.8 mM per liter. Mannitol excretion remained constant at about 0.29 mM per minute.

In Figure 5 the equilibrium values of the "creatinine" U/P corresponding to the three infusion rates of pitressin in Experiment 1 have been plotted against the dose expressed as mU per hour per kg. of body weight. On the same graph a curve from data obtained by Shannon[16] in a dog with experimental diabetes insipidus is shown, the infusion rate of pituitrin being likewise expressed per kg. of body weight. The similarity of form in the two curves indicates that the general relationship of the renal tubular response to increasing blood concentrations of antidiuretic hormone is not greatly different in the two species. Although the correspondence would certainly not always be as close as that shown, it does suggest that relative to body size the range of hormone secretion under ordinary conditions is roughly of the same order of magnitude in both.

The interesting fact that U/P increased in Experiment 2 (Fig. 2) after the pitressin infusion was terminated—during which time the blood concentration of pitressin must have been decreasing rapidly—makes advisable the comparison of the terminal portions of Experiments 1 to 3 shown in Figure 6. By chance, total solute excretions apparently were such that equilibrium U/P was lowest during the 295 and greatest during the 16 mU per hour infusions. Termina-

TABLE V*

EXPERIMENT 5, NOVEMBER 20, 1946

Period	Time	Cumulative Balance		Urine Flow (ml./min.)	Mannitol		Plasma Chloride Concentration (mM/L.)	Urine Concentration			Urinary Excretion		
		Water (ml.)	Chloride (mM)		U/P	Clearance (ml./min.)		Chloride (mM/L.)	Mannitol (mM/L.)	Cl × 2 + Mannitol (mM/L.)	Chloride (mM/min.)	Mannitol (mM/min.)	Cl × 2 + Mannitol (mM/min.)
	A.M.												
	8:40	Begin infusion I—NaCl (62 mM/L.) and mannitol (166 mM/L.)											
2	9:29– 9:45	1,016	−21.0	13.00	9.2	119	102.2	17.1	23.0	57.2	0.222	0.296	0.740
3	−10:00	998	−22.1	12.67	8.9	113		16.9	22.2	56.0	0.213	0.280	0.706
4	−10:18	942	−23.6	12.90	8.2	106	102.5	17.0	20.4	54.4	0.220	0.262	0.702
5	−10:31	933	−24.6	13.92	8.5	119		18.0	20.9	56.9	0.251	0.289	0.791
	10:25	Begin infusion II—30 mU of pitocin per hour in physiologic saline											
6	10:31–10:45	920	−25.4	13.57	9.7	131		20.1	23.2	63.4	0.272	0.312	0.856
7	−11:00	898	−26.6	13.40	10.6	142		21.9	25.2	69.0	0.293	0.337	0.923
8	−11:17	874	−27.7	12.11	10.7	130	102.4	23.2	26.2	72.6	0.281	0.316	0.878
9	−11:30	899	−28.4	10.85	10.6	115		23.1	25.9	72.1	0.251	0.285	0.787
10	−11:45	752	−29.4	11.48	10.9	125		23.9	26.4	74.2	0.274	0.301	0.849
11	−12:00	926	−29.9	10.54	10.7	113		23.9	25.6	73.4	0.252	0.269	0.773
12	−12:15	778	−30.9	11.80	10.8	127	102.7	23.8	25.3	72.9	0.281	0.296	0.858
	12:17	End infusion II											
13	12:15–12:31	765	−33.1	11.62	10.5	122		22.8	24.6	70.2	0.265	0.284	0.814
14	−12:43	633	−34.6	12.50	10.5	131		22.1	24.7	68.9	0.276	0.307	0.859
15	− 1:00	756	−36.5	11.53	10.7	123		21.6	25.4	68.6	0.249	0.291	0.789
16	− 1:17	567	−38.6	12.52	10.2	128	102.4	20.9	25.0	66.8	0.262	0.311	0.835
17	− 1:42	681	−40.7	9.03	10.3	93		20.1	25.1	65.3	0.181	0.225	0.587
	1:40	Begin infusion III—42 mU of pitressin per hour in physiologic saline											
18	1:42– 2:14	743	−41.8	4.94	28.6	141		56.7	67.3	181	0.280	0.331	0.891
19	− 2:44	908	−40.6	1.63	78.0	129	102.4	126	184	436	0.206	0.298	0.710
20	− 3:09	1,077	−39.0	1.40	84.9	119		129	202	460	0.181	0.281	0.643
	3:12	End infusion III											
21	3:09– 3:29	1,079	−39.6	1.37	84.9	116	101.6	135	205	475	0.185	0.278	0.648
22	− 3:50	1,077	−40.6	1.59	75.8	121		118	188	424	0.188	0.299	0.675
23	− 4:04	1,051	−41.3	3.28	36.2	119		57.5	92.3	107	0.188	0.301	0.677
24	− 4:19	1,011	−42.0	7.47	15.5	116		24.3	39.5	88.1	0.181	0.294	0.656
25	− 4:33	1,041	−42.5	10.00	11.5	115	99.8	17.1	29.3	63.5	0.171	0.291	0.633
26	− 4:41	1,105	−42.8	11.52	11.0	127		15.5	28.7	59.7	0.178	0.329	0.685
27	− 4:52	1,005	−43.3	11.04	10.3	114		15.5	27.2	58.2	0.171	0.298	0.640
	4:50	End infusion I											
		Integrated mean value 122											

* At 7:20 A.M. the subject drank 600 ml. of water but ate no breakfast. Balances were considered to be zero at 6:35 A.M. Cumulative balances were calculated as in Table I; 150 ml. of water were drunk approximately every seventeen minutes through period 19 and once during periods 25 and 26. Four 5 gm. quantities of lactose were eaten between 1:00 and 4:00 P.M. Infusion I ran continuously at an average rate of 2.24 ml. per minute from 8:40 A.M. to 4:50 P.M. Infusions II and III were administered at rates of 0.5 and 0.7 ml. per minute, respectively. The patient remained recumbent throughout except for some of the voidings.

tion of the infusion caused an almost immediate fall in U/P after the 16 mU per hour infusion (Exp. 3), indicating that on this day the blood concentration in equilibrium with this infusion rate was very near to the minimal concentration

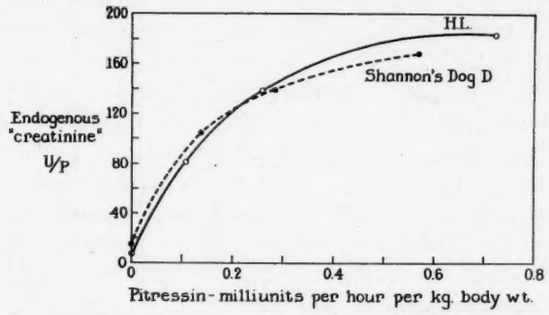

FIG. 5. Data of Exp. 1 (H. L.) compared to those obtained by Shannon[16] in a dog with diabetes insipidus which received pituitrin intravenously at the indicated rates (Shannon's Table 1). The U/P values shown are those corresponding to the final periods at the respective infusion rates, by which time the blood concentration of pituitrin is assumed to have reached equilibrium. Creatinine in the dog experiment was exogenous. Since the pitressin used in H. L. had probably deteriorated somewhat by the time of this experiment, the curve should properly be displaced to the left by about 16 per cent, a change which would not significantly alter the conclusion that the two curves are roughly similar.

capable of evoking maximum antidiuresis. The thirty-minute delay in the decline of U/P following the 50 mU per hour infusion (Exp. 1) presumably represents the time required for the supraminimal blood pitressin concentration to fall to that minimal value below which a decreasing concentration again resulted in a decreasing tubular water reabsorption. As the blood concentration fell following the 295 mU per hour infusion, U/P increased during the first hour to the same maximum level observed with the infusion of 50 mU per hour; thereafter the U/P decline is similar to the other two. The interrupted line represents the change in "creatinine" U/P following ingestion of 1,500 ml. of water in Experiment 4, time being measured from the beginning of drinking. Since drinking was not completed until twenty minutes later and since a considerable interval must have elapsed before the blood dilution was sufficient to arrest ADH secretion,[41,42] it is apparent that this curve should be displaced somewhat to the left to be strictly comparable with the three post-pitressin curves. Therefore, assuming endogenous ADH to be cleared from the blood at the same rate as pitressin, it may be inferred that the endogenous concentration at the beginning of water ingestion in Experiment 4 was equivalent to that which would be attained at equilibrium with an infusion of less than 50 mU and perhaps not greater than 16 mU per hour.

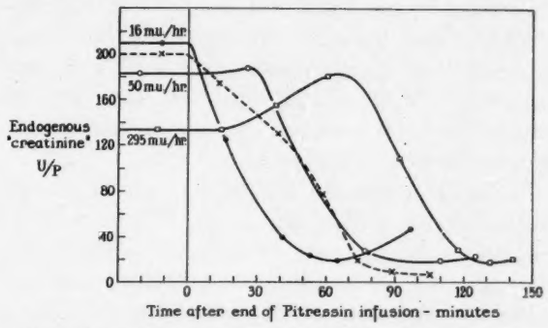

FIG. 6. Data from Exps. 1, 2, 3 and 4, showing time course of the "creatinine" U/P following termination of pitressin infusion which is indicated by zero on the time scale. The points marked *x*, connected by the interrupted lines, describe the changes following the ingestion of 1,500 ml. of water in Exp. 4, the beginning of drinking being considered as zero time. The irregularity of this curve is attributed to the interplay of three factors: (1) the increasing rate of solute excretion (see Fig. 4), (2) the fact that the posterior pituitary secretion is progressively inhibited as intestinal water absorption proceeds and (3) clearance of existing ADH from the blood. In the three post-pitressin curves the second factor is not involved. Due to deterioration of pitressin the infusion rates indicated in the graph should properly be decreased to approximately 12.3, 42 and 245 mU per hour, respectively. The course of the U/P in the post-pitressin periods of Exp. 4 is not shown here because the rate of chloride excretion was considerably higher than in Exps. 1 to 3.

Effects of Pitressin and Pitocin on Urinary Chloride Excretion. Because of conflicting data in the literature concerning the effects of posterior pituitary extracts on the rate of chloride excretion a closer examination of the data in the present experiments is warranted. In all but Experiment 2, in which inadvertently the urine specimens were discarded before chloride analysis could be done, the excretion rates during the pitressin infusions show no consistent change from the trends observed before and afterward. In Experiment 4 (Fig. 4) the rate of chloride excretion preceding the pitressin infusion was relatively high and was associated with the highest rates of urine flow ever observed in this subject. In this experiment the progressive antidiuresis during the pitressin infusion was associated with a decline in the excretion of chloride as well as of the other solutes. Whereas the extent to which the urine flow decreased under the influence of this rather high rate of

pitressin administration is assumed to be determined by the rate of total solute excretion, it is possible that the latter was, in turn, somehow limited by the antidiuresis. Such a phenomenon has been well established for urea[43–45] and is illustrated by the data in Table IV and Figure 4. Holden and Bulger[46] observed the same phenomenon when they extended Dole's concept to a variety of solutes; however, they did not study the excretion of the major endogenous electrolytes as functions of V.

It is clear that in the subject of the present experiments physiologic rates of pitressin administration had no chloruretic action, even though the immediately preceding rates of chloride excretion varied from as low as 0.12 mM per minute to as high as 0.39 mM per minute. Nevertheless, it remains possible that in Experiment 2 such an effect may have occurred. The only reasonable explanation for the fact that the "creatinine" U/P did not rise above 134 or that the urine flow did not fall below 0.9 ml. per minute by the end of more than 2.5 hours of infusion at 295 mU per hour is that the concurrent rate of total solute excretion was relatively high (cf. Exp. 4). The rise in U/P following termination of the infusion strongly suggests that a progressive decrease in the rate of solute excretion occurred, which facilitated a progressively increasing tubular water reabsorption under the influence of the pitressin remaining in the blood. Since the patient had resumed mild activity in the upright posture during the post-pitressin periods, it is not unlikely that NaCl excretion decreased as it had in the corresponding phase of Experiments 1 and 3. Whether the postulated increased rate of solute excretion during the pitressin infusion was an effect of the extract or was spontaneous cannot be determined. It is assumed that maximum antidiuresis was present not only during the last infusion period but also during the three subsequent periods, the variation in V and U/P being the result of variation in total solute excretion.

In this connection it should be noted that Shannon's dog experiments[16] showed considerable variation in the chloride excretion among the four dogs used and from one experiment to another in any one dog, but within a given experiment an increased rate of extract infusion usually resulted in increased chloride excretion. These increases were independent of the GFR since this usually remained constant. The extract used by Shannon was the unfractionated product, pituitrin; it is not certain, therefore, which of the two fractions, antidiuretic or oxytocic, was responsible for the chloruretic effect in his experiments.

Early in the present investigation an attempt was made to study the influence of pitocin on chloride excretion. The results are summarized in Table VI (Exp. 6, November 11, 1946). As in the previously described Experiment 5 a constant infusion of mannitol was maintained throughout, keeping the plasma concentration between 2.4 and 2.7 mM per L. The procedure was similar to that in Experiment 5 except that no NaCl was incorporated in the infusion; this resulted in a substantially negative chloride balance by the end of the study.

The priming dose of 100 mU of pitocin followed by a sustaining infusion at the rate of 48 mU per hour had no discernible effect on chloride excretion, although the antidiuretic content of the extract (1 pressor unit to each 20 units of oxytocic substance) was sufficient to induce a moderate rise in the mannitol U/P to 56.8 as the urine flow decreased to 2.08 ml. per minute.

In this subject, therefore, at least under the conditions employed, neither pitressin nor pitocin had any chloruretic effect.*

Influence of the Rate of Solute Excretion on the Response of Urine Flow to Pitressin Administration. It was pointed out in the introduction that the extent of antidiuresis is related to the rate of total solute excretion.[21–24] Since under the conditions of these authors' studies, i.e., infusion of hypertonic solutions during hydropenia, antidiuretic hormone activity may be assumed to have been maximal, it follows that the minimum urine flow (maximum U/P) varies as a direct function of the rate of total solute excretion (milliosmols per minute). It is of interest that this relationship is apparent even in the present experiments in which the solute excretion rates are low compared to most of those studied by the investigators just cited. Although freezing point measurements were not made, limited

* From the observations of Grollman and Woods[47] that oxidation with H_2O_2 destroys the chloruretic activity (for rats) of commercial pituitrin without altering the antidiuretic activity, it is possible that some inactivation had occurred in the ampoules of pitressin used in this study. This would seem to be unlikely, however, at least in Exp. 5, since this was performed within six weeks after the date of the manufacturer's original assay of this lot of pitressin. The possibility remains that other lots of pitressin might have been chloruretic in this subject.

TABLE VI

EXPERIMENT 6—NOVEMBER 11, 1946

Period	Time	Cumulative Balance		Urine Flow (ml./min.)	Mannitol		Urea Clearance (ml./min.)	Plasma Chloride Concentration mM/L.)	Urine Concentration				Urinary Excretion			
		Water (ml.)	Chloride (mM)		U/P	Clearance (ml./min.)			Chloride (mM/L.)	Mannitol (mM/L.)	Urea (mM/L)	Cl × 2 + Mannitol + Urea (mM/L.)	Chloride (mM/min.)	Mannitol (mM/min.)	Urea (mM/min.)	Cl × 2 + Mannitol + Urea (mM/min.)
	A.M.															
0	7:08– 8:45			0.94			52.1	108.2	94.9		318		0.089		0.298	
	8:55	Begin infusion I—Mannitol (274 mM/L.)														
1	9:36–10:09			1.94	65.1	126	61.5	106.5	93.7	159	183	530	0.182	0.309	0.337	1.010
2	–10:39	731	− 6.0	2.17	58.1	126	67.1		91.6	144	178	505	0.199	0.311	0.386	1.095
3	–10:58	831	−12.0	4.05	29.1	118	72.7		81.2	70.0	103	335	0.329	0.283	0.417	1.358
4	–11:20	806	−18.7	9.07	13.5	122	80.7		32.9	33.2	50.2	149	0.298	0.301	0.455	1.352
5	–11:39	691	−23.7	11.18	10.0	118	78.2	105.4	24.1	26.8	38.6	114	0.269	0.289	0.432	1.259
6	–11:49	637	−26.9	14.30	10.0	142	94.4		22.6	24.0	35.9	105	0.323	0.343	0.515	1.503
7	–12:01	670	−30.0	11.35	10.3	116	73.6		22.1	24.9	35.1	104	0.251	0.282	0.398	1.183
8	–12:12	641	−32.7	11.24	10.3	116	76.0		22.6	24.8	36.1	106	0.254	0.287	0.406	1.193
9	–12:27	568	−36.5	10.73	10.4	111	71.1	103.8	23.8	26.3	35.0	109	0.255	0.283	0.376	1.168
10	–12:41	588	−40.1	11.13	11.1	124	79.9		23.0	29.1	37.2	112	0.256	0.324	0.414	1.250
11	–12:59	501	−44.5	9.94	11.3	113	75.3		24.5	30.8	38.6	118	0.243	0.305	0.384	1.176
	1:06	100 mU Pitocin I.V.; begin infusion II*—Mannitol (274 mM/L.) with Pitocin—48 mU per hour														
12	12:59– 1:17	562	−48.8	6.45	16.1	104	66.8		37.4	43.6	51.5	170	0.241	0.281	0.332	1.095
13	– 1:38	692	−53.6	2.28	50.6	115	58.6	103.5	100.6	128	125	454	0.229	0.305	0.285	1.035
14	– 2:17	966	−61.6	2.08	56.8	118	62.6		99.0	150	143	491	0.206	0.306	0.298	1.021
	2:30	End infusion II, resume infusion I*														
15	2:17– 2:38	1,011	−65.7	2.28	46.0	105	62.7	102.7	86.0	123	127	422	0.196	0.277	0.290	0.956
16	– 3:08	904	−71.4	3.93	30.6	120	78.4		48.0	77.7	92.8	266	0.189	0.305	0.365	1.047
17	– 3:28	806	−74.2	5.30	19.7	104	66.8		26.2	49.4	58.8	161	0.139	0.262	0.312	0.852
18	– 3:44	692	−76.7	7.50	16.2	122	73.6		21.1	40.6	46.0	129	0.158	0.305	0.345	0.967
19	– 3:56	602	−78.5	7.83	15.8	123	74.8	102.5	19.5	38.4	45.0	122	0.153	0.301	0.352	0.958
	4:10	End infusion I														
		Integrated mean value 118														

* The moment that the infusion began and stopped entering the vein is not definitely known in this experiment because infusions were switched at the top of the tubing without first draining the previous solution from the tubing as was done in Experiments 1 to 5. The times indicated above are estimates based on the rate of the infusion and the volume of tubing. The actual change-over of the infusion flasks occurred fifty minutes prior to the times given above. Because of mixing and diffusion some pitocin probably reached the blood stream during periods 10 and 11, and for the same reason the pitocin infusion entering during the latter part of period 14 may have been somewhat diluted. The priming dose of 100 mU of pitocin was given at 1:06 which was the estimated time of arrival of infusion II at the vein.

No water was taken by mouth since the evening before and until period 2. Balances were considered to be zero at the beginning of period 2. Cumulative balances were calculated as in Table I. Water was drunk at the rate of 85 ml. every ten minutes during periods 2 through 15 and none was drunk thereafter. No food was taken before or during this experiment. The infusions contained no NaCl. The average rate of administration of the three infusions was 1.18 ml. per minute. The patient remained recumbent throughout. The initial and final weights were both 69.6 kg.

inferences can be drawn concerning the magnitude of the total solute excretion and urinary concentration from the sum of several determined solutes comprising well over half of the total.

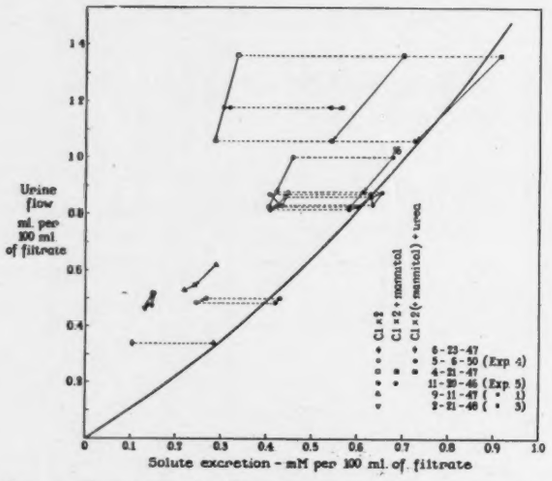
Urine flow ml. per 100 ml. of filtrate
Solute excretion - mM per 100 ml. of filtrate
Cl x 2
Cl x 2 + mannitol
Cl x 2 (+ mannitol) + urea
6-23-47
5- 6-50 (Exp 4)
4-21-47
11-29-46 (Exp 5)
9-11-47 (" 1)
2-21-48 (" 3)

FIG. 7. Data from periods assumed to represent maximum antidiuresis. Urine flow per 100 ml. of glomerular filtrate is related to the solute excretion per 100 ml. of filtrate. The smooth curve was fitted by the method of least squares to the data designated by the solid symbols and represents the equation:

$$V/C = \frac{U_s V/C}{0.706e^{-0.837U_s V/C} + 0.310}.$$

The symbols have the same meaning as in equation 2 of the text except that U_s here is the sum of twice the chloride plus the urea (plus mannitol when present), and that C represents the clearance of either endogenous "creatinine" or of mannitol. The abscissal value of the open symbols indicates twice the rate of chloride excretion; the half-solid symbols, the sum of twice the chloride plus mannitol excretion; and the solid symbols the sum of these plus urea. The data correspond to the following periods from experiments detailed in the present report: Exp. 4, periods 1 and 2, and 16–24; Exp. 5, periods 20 and 21; Exp. 1 periods 15–17; and Exp. 3, periods 14–19.

In Figure 7 the smooth curve represents the best fit, by the method of least squares, to all the available data obtained during maximum antidiuresis in this subject for periods in which the excretion both of chloride and urea were measured. The equation used for fitting this curve is a modification of that of Rapoport et al.[23,24] Their equation represents the solution of a differential equation based on the fundamental assumption that the concentration of total solutes in the urine, under the condition of hydropenia, approaches the plasma concentration of total solutes asymptotically as the total solute excretion increases.* With a change of symbols their equation 3 may be written:

$$1.\ U_s = A'e^{-kU_s V} + B.$$

Dividing both sides into $U_s V$ gives:

$$2.\ V = \frac{U_s V}{A'e^{-kU_s V} + B}$$

which is identical to their equation 5. U_s is the total solute concentration in the urine in osM per L., V is the urine flow in ml. per minute, A′ and k are constants, and B is the plasma total solute concentration in osM per L. The equation used for the curve in Figure 7 is the same as equation 2 except that U_s indicates the sum of twice the chloride plus urea (plus mannitol, when present), that the plasma concentration is assumed to be 0.31 osM per L., and that V is divided by the observed "creatinine" clearance (or mannitol clearance). This modification assumes that the urine flow is related to the excretion of urea plus NaCl (estimated as twice the chloride) by the same function as for total solute excretion, only the constants being different. Furthermore, dividing by GFR eliminates errors in V due to incomplete bladder emptying and changes the relationship to the more fundamental one between the solute excretion per 100 ml. of GFR and the percentage of filtered water excreted.

The solid symbols in Figure 7 represent the data to which the curve was fitted. They correspond to periods 1 and 2 and 16 through 24 of Experiment 4 and to three periods from two experiments not described in this report. The distance along the abscissa from zero to the open symbols represents the value for twice the chloride excretion. The horizontal distance from the open to the half-solid symbols represents mannitol excretion where this solute was present. In these experiments the horizontal distance from the half-solid to the solid symbols indicates urea excretion. In the others urea excretion is measured by the interval between the open and solid symbols. Additional points are shown from Experiments 1, 3 and 5 in which urea was not measured. These are indicated by open (and

* Their assumption appears to be validated by the observations of Wesson, Anslow and Smith[48,49] and of Mudge, Foulks and Gilman[50] that during extreme osmotic diuresis the osmolar concentration of urine is approximately equal to the concurrent osmolar concentration of the plasma.

half-solid) symbols not connected by interrupted lines to any solid symbols. Points from successive periods are connected by solid lines.

In all of the periods shown it is assumed that for all practical purposes the minimum urine flow possible for the given rate of solute excretion had been attained and that the blood concentration of antidiuretic hormone was at or above the minimum required to induce the maximum possible antidiuresis. The data shown comprises: three early morning periods of spontaneous antidiuresis following nine to twelve hours of fluid deprivation (6-23-47 and periods 1 and 2 of Exp. 4); two periods (4-21-47) during mild hydropenia, beginning seventy minutes after the intravenous injection of 100 ml. of 25 per cent mannitol and three minutes after the subcutaneous injection of 10,000 mU of pitressin; and all the rest were obtained during and after intravenous infusions of pitressin while the subject was hyperhydrated.

It is seen that maximum antidiuresis in those experiments in which mannitol was absent was manifested by urine flows as low as 0.34 ml. to as high as 1.0 ml. per 100 ml. of glomerular filtrate. This range corresponds to solute excretions (twice the chloride plus urea) of 0.29 to 0.68 mM per 100 ml. of glomerular filtrate. These values represent spontaneous solute excretion rates for this subject after overnight dehydration and during hyperhydration, respectively. It is evident that the equation based on that of Rapoport[24] reasonably well describes the relationship between the excretion of these solutes and the minimum urine flow.

The data from Experiments 1 and 3 in which only chloride was measured are probably adequate, as plotted in Figure 7, to explain why in Experiment 3 the minimum urine flow was lower under the influence of only 16 mU of pitressin per hour than in Experiment 1 under 50 mU per hour. These respective infusion rates had presumably resulted in blood pitressin concentrations practically equal to and greater than the minimum required to reduce urine flow to the lowest possible values. (See also Fig. 6.) However, the lower chloride (and presumably lower total solute) excretion in Experiment 3 permitted the urine flow to fall lower than in Experiment 1.

Relationship between Total Solute Excretion and the Minimal Blood ADH Concentration Required for Maximum Antidiuresis. The question as to what minimal blood ADH concentration is needed to induce maximum antidiuresis at any given rate of total solute excretion cannot be answered from the present studies. However, it is certain from the observations of Hare, Hare and Phillips[18] that much greater concentrations of ADH are required when the solute excretion is very high (as in osmotic diuresis) than when it is in the usual range. Although the precise nature of this relationship is not known, it seems logical to infer that the minimal blood ADH concentration would be a continuously increasing function of the total solute excretion rate.

In this connection it is pertinent to re-examine one of the important experiments of Hare et al.[18] in the light of the concepts subsequently developed by Rapoport and associates.[23,24] These permit a more exact estimation of the rate of ADH liberation in osmotic diuresis than was possible at the time of the original publication in 1943 and of the subsequent paper of Chambers, Melville, Hare and Hare[19] in 1945. Figure 8 shows data recalculated from Table II of Hare et al.[18] concerning four experiments on Dog S with experimental diabetes insipidus. In each experiment 2.5 per cent NaCl was infused intravenously at the rate of 10 ml. per minute for forty-five minutes, beginning at the onset of the periods marked 1. Urine collections were made every fifteen minutes; creatinine clearance was used to measure the GFR. In one experiment only the hypertonic saline was administered while in the other three pitressin was included in the infusion in concentrations such that the rates of administration were 60, 180 and 720 mU per hour during the forty-five-minute period. In each experiment the GFR increased progressively during the saline administration, accompanied by an increasing chloride excretion; there was no discernible difference among the four experiments in the relationship between GFR and chloride excretion. Since the GFR changed during the studies, both urine flow and chloride excretion have been divided by the concurrent creatinine clearance in Figure 8. Because of the design of the experiments the data are of a transitional rather than a stationary character, requiring that temporal changes be taken into account as was done by the authors. As the infusion proceeded the blood concentration of chloride and pitressin both were increasing rapidly. Although the latter probably had not reached a true equilibrium state during the last fifteen minutes of the infusion (points marked 3), it nevertheless must

have been closely approaching a constant and maximum value by this time.

In the absence of pitressin the urinary chloride concentration increased progressively with time after the onset of the infusion (the reciprocal of the slope of a line drawn from the origin through

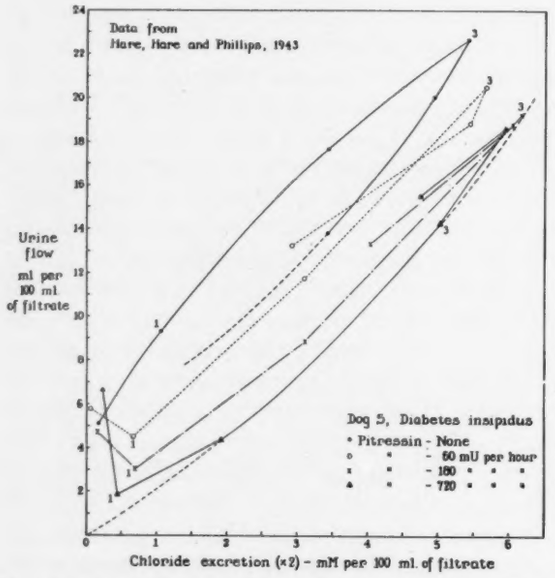
Data from
Hare, Hare and Phillips, 1943
Urine flow
ml per 100 ml of filtrate
Dog 5, Diabetes insipidus
• Pitressin - None
○ " - 60 mU per hour
× " - 180 " " "
▲ " - 720 " " "
Chloride excretion (×2) - mM per 100 ml. of filtrate

FIG. 8. Data recalculated from Table II of Hare, Hare and Phillips.[18] The hypertonic saline infusions began with the periods marked 1 and continued through the next two periods. The pitressin indicated on the graph was mixed with the hypertonic saline. Smooth curves have been drawn by eye through the ascending and descending solid circles from the experiment in which no pitressin was given. The descending portion is extended as an interrupted line on the assumption that the curve would have returned toward the control value in a smooth fashion. Another smooth curve was drawn from the origin through the second and third infusion periods of the 720 mU per hour experiment (solid triangles) and extended through the third infusion period of the 180 mU per hour experiment (*x*). This curve is assumed to be the locus, relative to the rate of NaCl excretion, of the minimum urine flow attainable in this dog under maximum antidiuretic hormone influence. By this reasoning 180 mU per hour of pitressin was sufficient to induce maximum antidiuresis at the time of the greatest chloride excretion. Infusion of 60 mU per hour for forty-five minutes was clearly insufficient.

a given point equals two times the urinary chloride concentration in mM per ml.). The maximum reached by the last period of this experiment was 124 mM per L. The hysteresis during saline administration displayed in this curve has been repeatedly observed, for example, by Shannon[51] in a dog with diabetes insipidus (Shannon's Table VI), by Hare et al.[18] in a normal dog (Hare's Table I), by Hickey and Hare[20] in normal man (their Table I) and by Rapoport and associates.[26] The phenomenon does not appear to depend on changes in GFR. Since it occurs in the absence of ADH, it cannot be attributed primarily to changes in the rate of ADH liberation during the course of such an infusion. It may represent an intrinsic renal tubular property or conceivably might be due to a change in the rate of liberation of salt-active adrenal corticoids.

Absence of ADH does not prevent the renal tubules from responding to plasma hyperosmolarity by increasing the urine osmolarity. The diuresis which proceeds in the absence of ADH increasingly acquires an "osmotic" character as the chloride excretion becomes higher. The difference between this curve of urine flow and that under the influence of 720 mU per hour of pitressin becomes proportionately smaller as chloride excretion increases.

Infusion of pitressin at the rate of 720 mU per hour during the forty-five-minute saline infusion evidently produced blood concentrations of antidiuretic substance equal to or greater than the minimal concentration required to induce the maximal antidiuresis possible for the concurrent rate of solute excretion (twice the chloride excretion may be assumed to account for more than two-thirds of the total solutes in most of the points shown in Figure 8). That this was already the case in the second period (15th to 30th minute) of the infusion is evidenced by the location of this point (solid triangle) on the smooth curve passing through the origin. This curve is assumed to define the biologic limit of minimum urine flow relative to the chloride (plus sodium) excretion. Since the point corresponding to period 4 (0 to 15 minutes after the end of the infusion) was still almost on the curve despite the rapid blood clearance of pitressin, the blood concentration during period 3 is considered to have been supraminimal. More convincing evidence of this is the fact that both periods 3 and 4 from the 180 mU per hour experiment fell on the same curve, indicating that this lower rate of infusion produced an effect equivalent to that of the higher rate.

If it be accepted that the blood pitressin concentration in each experiment had practically reached equilibrium by period 3, the curves in Figure 8 indicate that by this time a normal dog with a comparable rate of chloride excretion and of glomerular filtration would

have attained a blood concentration of secreted ADH approximately equivalent to that in equilibrium with an infusion of 180 mU of pitressin per hour, or possibly even less, inasmuch as the response of the first post-infusion period in this experiment was still on the curve of maximum antidiuresis.

These data cannot validly be compared with similar measurements obtained in the normal dog L of Hare, Hare and Phillips[18] (their Table 4) because of the difference in the rate of chloride excretion during and after infusion of 2.5 per cent NaCl at 10 ml. per minute. As was clearly stated in the subsequent paper of Chambers, Melville, Hare and Hare,[19] the creatinine U/P ratio (or urine flow per 100 ml. of filtrate) can be used as an index of pituitrin activity to compare a normal dog releasing ADH under the stimulus of infused hypertonic saline with a dog with diabetes insipidus receiving pituitrin during the same kind of infusion only in those experiments in which the rates of glomerular filtration and solute excretion are comparable from period to period. To these restrictions we would add the factor of differences in the individual renal responsiveness to any given blood ADH concentration. The best comparisons, therefore, are those made among different experiments in the same animal, as these authors did in the case of their Dog S.

On the basis of the reasoning presented in the previous two paragraphs we believe that, contrary to the authors' conclusions,[19] infusion of 720 mU of pituitrin per hour not only provided a full normal replacement for Dog S under these conditions of plasma hyperosmolarity but actually must have been somewhat excessive. This interpretation of these important data may account wholly or in part for the failure of Chambers et al.[19] to demonstrate endogenous ADH in 40 to 50 ml. of peripheral venous blood of normal 15 kg. dogs withdrawn at the termination of a forty-five-minute infusion of 2.5 per cent NaCl at 10 ml. per minute when, by their assay method, as little as 1.0 mU of pitressin per 100 ml. was detectable.

Graphed in the manner of Figure 8 the data of Hickey and Hare[20] obtained in a normal subject and a patient with diabetes insipidus in studies analogous to the dog experiments suggest that the infusion of 532 mU of pitressin per hour (patient L. S., 40 kg., diabetes insipidus) during a forty-five-minute infusion of 2.5 per cent NaCl at 10 ml. per minute produced a blood concentration of pitressin by the third period (30th to 45th minute) considerably in excess of the minimum required for maximum antidiuresis. In these experiments the relatively lower rate of saline infusion induced a smaller increase in plasma chloride and therefore was a less powerful osmotic stimulus than in the dog experiments.

The replacement infusions of posterior pituitary extract, as used by Shannon,[16] by Hare and co-workers[18–20] and by the present author, provide a measure of the *rate of liberation* of endogenous ADH in various conditions. For example, if a sustained urine flow of 1 ml. per minute, occurring during mild dehydration under the influence of endogenous ADH, is reproduced after heavy water-loading in the same subject (GFR and total solute excretion held constant) by a constant infusion of pitressin which has run long enough to produce a constant blood ADH concentration, it could be concluded that the posterior pituitary had secreted ADH at a rate equivalent, in milliunits per hour, to that of the pitressin infusion. This conclusion implies that the blood clearance (ml. per min.) is the same for endogenous ADH as for the infused pitressin. A more precise conclusion, therefore, would be that the equilibrium blood pitressin concentration was equivalent in antidiuretic activity to the blood ADH concentration occurring in such a spontaneous submaximal antidiuresis.

However, such infusion experiments do not in themselves indicate actual blood ADH *concentrations*. The concentration reached at equilibrium is necessarily related by some unknown function directly to the rate of pitressin infusion and inversely to the blood ADH clearance (total clearance, by inactivation and diffusion, as well as by renal excretion). Since the magnitude of this clearance and whether or not it is constant in a given subject are not known, it is not possible at present to estimate blood concentration from the infusion rate alone.

Experiments of Marx[52] together with those of Hare, Hickey and Hare[53] and Chambers et al.[19] make possible a rough estimate of ADH concentration in the peripheral blood of normal dogs during dehydration. Marx withdrew 100 ml. of blood from a thirsting 19.5 kg. dog and reinjected it after the animal had been hydrated. A small but unequivocal decrease in urine flow promptly resulted. Blood withdrawn during water diuresis had no effect on the diuresis when re-injected. The antidiuretic response unfortu-

nately was not calibrated in terms of response to standardized posterior pituitary extract.

On the other hand, Hare and associates failed to get positive assays when 10 to 50 ml. volumes of external jugular blood, obtained from normal dogs after two to four days of water deprivation[53] or after infusion of hypertonic saline,[19] were assayed directly in dogs with experimental diabetes insipidus. Their method, capable of detecting as little as 0.1 mU of pitressin, was highly controlled and not open to criticisms such as were applied by these authors,[19] as well as by Gilman and Kidd,[54] to the observations of Melville[55] who found apparent concentrations of as much as 10 mU per 100 ml. in blood subjected to an extraction procedure. Estimating the antidiuretic effect observed by Marx[52] as being equivalent to that induced by injection of about 0.1 or 0.2 mU of pitressin, it may be concluded tentatively that the peripheral blood ADH concentration in dogs deprived of water three to ninety-six hours is definitely less than 1 mU and perhaps no greater than about 0.1 mU per 100 ml. at a time when the kidneys are exhibiting maximum antidiuresis.

More recently Ames, Moore and Van Dyke[56] have demonstrated in normal dogs that the intracarotid infusion of 0.5 M NaCl at 6 ml. per minute stimulated ADH liberation to such an extent that after about thirty minutes of infusion the external jugular blood had reached a concentration of about 10 mU per 100 ml. when assayed by a method similar to that of Hare and associates.[53] At the same time the internal jugular blood contained approximately 50 mU per 100 ml. In a single experiment under similar conditions femoral vein blood was said to have contained no detectable hormone, a circumstance not readily explained solely by the dilution of the internal jugular blood flow in the total blood flow (cardiac output) since such a dilution factor would hardly reduce the concentration from 50 mU to less than 1 mU per 100 ml. If confirmed, this large decrease in ADH concentration from the internal jugular vein to the femoral vein would imply a rapid loss of activity in passing through the extremities due to diffusion or inactivation.

The contribution by Ames et al.[56] is especially important because positive antidiuretic assays of blood were obtained by an extremely well controlled technic. They provide a measure of what must approach the maximum possible rate of sustained ADH liberation by the normal neurohypophyseal system in dogs. For this reason, unfortunately, these measurements have little bearing on the rates of liberation or blood concentrations occurring under ordinary conditions. The local increase in osmolarity of arterial blood reaching one-half of the osmoreceptors in the experiments of Ames et al.[56] was far greater than the estimated 1.8 per cent increase which Verney found caused the release of about 3.6 mU per hour.[17] It also must have been many times greater than that resulting from the two- to four-day periods of dehydration employed by Hare, Hickey and Hare[53] and at least several times higher than that attained during the *intravenous* infusion of hypertonic NaCl,[18,19] under both of which conditions the *external* jugular blood ADH concentration was less (perhaps much less) than one-tenth of that found by Ames et al.[56] It may be accepted, therefore, that simple dehydration in dogs leads to concentrations no greater and perhaps considerably less than 1 mU per 100 ml. Such seemingly low concentrations, nevertheless, are sufficient to induce maximum antidiuresis in the presence of the relatively low solute excretion rate obtaining in this condition.

Only Page[57] and Brun, Knudsen and Raaschou[58,59] appear to have used the direct transfer technic for estimating the ADH content in the blood of human subjects. Transfusion of 500 ml. of citrated peripheral venous blood from normal and eclamptic women into hydrated recipient women resulted in slight antidiuresis in some of the cases in both groups.[57] The state of donor hydration was not reported and the assay procedure was much less refined than in the animal studies cited.[18,19,53,56] Brun and associates,[58,59] however, obtained moderate but unequivocal antidiuresis in well hydrated recipients by infusion of 200 to 450 ml. of heparinized peripheral venous blood withdrawn from hydrated donors in whom brief syncope had been induced a few minutes earlier by passive tilting. Comparable transfusions from control hydrated donors had no antidiuretic effect in all uncomplicated experiments. Unfortunately, the response of the recipients was not calibrated in terms of standardized posterior-pituitary extracts. Accurate estimates of the donor blood ADH concentrations cannot, therefore, be made. It may be surmised, however, from the data of Page and of Brun et al. that the strong neurogenic stimulation of the neurohypophyseal system during syncope liberated ADH at a

rate exceeding that resulting from moderate dehydration.

Role of the Glomerular Filtration Rate in Determining Urine Flow during Antidiuresis. In the foregoing comments it has been pointed out that the minimum urine flow under the influence of an excessive blood ADH concentration is determined by the rate of total solute excretion.[18,21–24] It has been inferred that the minimal hormone concentration in the blood needed to induce such maximum antidiuresis is likewise some direct function of the total solute excretion. Another important determinant of the minimum urine flow is, of course, the GFR itself.

As was pointed out in the reviews by Smith[27] and by Pitts and Sartorius,[7] urine flow will vary in proportion to GFR under conditions of maximum antidiuresis. That this generalization may be true only for some limited range of variation in GFR is suggested by the observations of Shannon.[51] He showed that dehydration in dogs with diabetes insipidus resulted in the elaboration of moderately hypertonic urine. Since these dogs secreted little or no ADH, this hormone possibly was not necessary for the process. However, reduction in the GFR was apparently an essential factor. The data[51] from these experiments (Shannon's Table II) have been recalculated and are shown in Figure 9, plotted in the manner of Figures 7 and 8. It is seen that the relationship between the excretion of total solutes per 100 ml. of filtrate and the urine flow per 100 ml. of filtrate after eighteen hours of dehydration is quite different from that during drinking *ad libitum* and after forced hydration. The tabulations in the Figure indicate the corresponding values of the creatinine clearance (C_{cr}) and of urinary milli-osmolar concentration (U_{mosM}). It is evident that, even in the apparent absence of ADH, varying the GFR alters the relationship between the minimal urine flow and the solute excretion, presumably by an intrinsic tubular mechanism. Addition of ADH to the blood in a supraminimal concentration, leaving the observed filtration rates unaffected, would be expected to displace these curves in a clockwise direction, perhaps to bring them nearer together but not to superimpose them. It may be conjectured on the basis of this expectation that under maximum ADH influence the urinary osmolar concentration, for a given rate of total solute excretion per 100 ml. of filtrate, would vary as some undefined inverse function of the GFR.

Some support for this assumption is provided by data from another of Shannon's experiments[16] (Shannon's Table II). In this experiment a dog with diabetes insipidus was given 20 mU of pituitrin per hour by continuous intravenous infusion. In Figure 10, the point marked 3 represents data from period 3 which began thirty-two minutes after the onset of the pituitrin infusion. Immediately after period 4, and thirty minutes before the beginning of period 7, 50 ml. of water per kg. were administered by stomach tube. The GFR increased from 26.3 ml. per minute in period 4 to 35.0 ml. per minute in period 7 and remained in the range of 35.0 to 38.8 ml. per minute until the pituitrin infusion was terminated after period 13. The smooth curve through the points from periods 7 through 13 was fitted by the method of least squares in the manner outlined in relation to Figure 7. On the basis of the reasoning of Rapoport et al.[23,24] it is assumed that this curve describes the relationship between the urinary total solute excretion and V, corresponding to the elevated GFR. That the antidiuresis was actually maximum during the post-hydration periods, as well as during

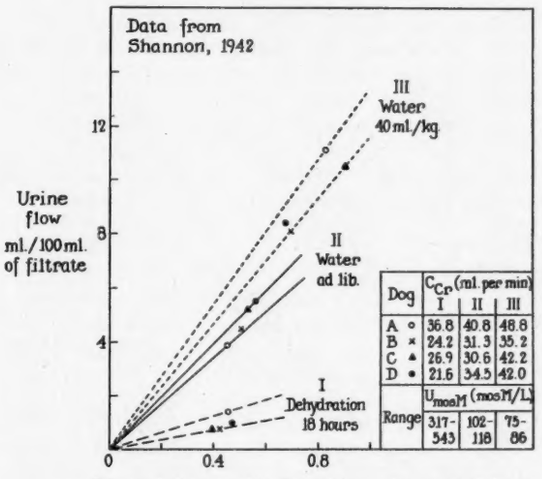

FIG. 9. Data recalculated from Table II of Shannon.[51] Total solute excretion was calculated from the freezing point measurements using the factor of 1.85 osmols per L. for each degree (C.) of freezing point depression. The points are connected to the origin by straight lines instead of curves of the type shown in previous figures because only a single value was given for each dog in each of the three experimental conditions. Note especially that the reduction in GFR after dehydration is associated with a marked displacement of the curve, whereas elevation of the GFR after forced hydration is associated with a lesser displacement in the opposite direction.

periods 3 and 4, is suggested by the fact that the antidiuresis persisted undiminished during the sixteen-minute post-infusion period (period 14 of Shannon's Table). Therefore, the vertical distance between the values from periods 3 and 4 and the smooth curve is an approximate

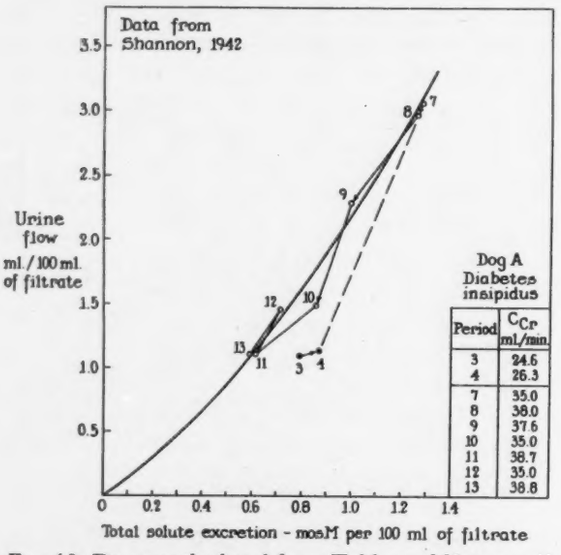

FIG. 10. Data recalculated from Table II of Shannon.[16] The points represent measurements made during a constant pituitrin infusion at the rate of 20 mU per hour. Immediately after period 4 the dog was given 40 ml. of water per kg. by stomach tube. The smooth curve through points from periods 7–13 was fitted by the method of least squares. Note that the elevation of GFR was associated with a counterclockwise displacement of the curve relating urine flow and total solute excretion. Since antidiuresis was maximum in periods 7–13 as well as in periods 3 and 4, it is assumed that the displacement is due to the change in GFR.

measure of the displacement of the relationship which apparently must be attributed to the change in GFR. In other words, for a given rate of total solute excretion the maximum urine osmolarity decreases as the GFR is increased.

The observations of Dancis, Birmingham and Leslie[60] on a polyuric child whose renal tubules were completely refractory to pitressin are in accord with the results of Shannon's experiments. They found that dehydration of twelve hours resulted in a greatly decreased urine flow and an increase in urine specific gravity from 1.004 before dehydration to the slightly hypertonic value of 1.016.

Obviously, additional experiments specifically designed for the purpose are needed to elucidate this effect of change in the GFR but Shannon's studies clearly indicate that variation in the GFR is one factor determining the proportion of filtered water which is excreted.

CONCLUSIONS

In brief, then, the urine flow per 100 ml. of glomerular filtrate and its inverse, the inulin U/P ratio (and, approximately, the U/P of "creatinine" or mannitol), are determined by at least four factors: (1) the rate of glomerular filtration, (2) the rate of total solute excretion, absolutely, as well as relative to the GFR, (3) the blood concentration of ADH and (4) the inherent responsiveness of the renal tubules to ADH, which may be altered by other endocrine influences[6,61] or by diseases such as chronic glomerulonephritis.[62]

It is evident that experiments devised to prove that ADH participates in the pathogenesis of edema in various diseases should take into account all four of these determinants of urine flow. In addition, it should be shown that substances excreted in the urine which are antidiuretic in test animals are in fact antidiuretic in man. There is an evident need for carefully controlled assays, by the direct transfer technic, of the blood ADH concentration in patients with these diseases as well as in normal subjects in various degrees of hydration.

SUMMARY

The rate of liberation of the neurohypophyseal antidiuretic hormone (ADH) was estimated in a normal 70 kg. man from the decrease in urine flow and rise in endogenous "creatinine" U/P ratio at the end of two-hour constant intravenous infusions of commercial pitressin, administered during a state of sustained hydration. Administration of as little as 7.5 milliunits (mU) per hour reduced the urine flow to 1.6 ml. per minute. Infusion of 16 mU per hour was sufficient to induce maximum antidiuresis by the end of two hours. On another occasion 50 mU per hour resulted in maximum antidiuresis; but because the effect persisted undiminished for more than one hour after cessation of the pitressin infusion, it is evident that the blood pitressin concentration achieved was greater than the minimum concentration required for maximum antidiuresis. The normal rates of ADH secretion under ordinary conditions of diet and hydration are equivalent, therefore, to intravenous pitressin infusions in the range of from less than 7.5 mU per hour to something under 50 mU per hour. This range of rates, per kg. of body weight,

corresponds approximately with that observed by Shannon in dogs with experimental diabetes insipidus. Chloruresis did not result from pitressin administration in any of these experiments.

Problems involved in estimating the rate of ADH secretion have been discussed. Certain important experiments in the literature were reexamined in detail to indicate the importance of the several factors in addition to antidiuretic hormone which determine the rate of urine flow.

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Studies of the Serum and Urine Constituents in Patients with Cirrhosis of the Liver during Water Tolerance Tests*

ELAINE P. RALLI, M.D., STEPHEN H. LESLIE, M.D., GEORGE H. STUECK, JR., M.D.†
and BERTRAM LAKEN, B.S.

New York, New York

A DISTURBANCE in water balance as reflected by a diminished rate of urine excretion has long been associated with clincially severe liver damage.[1,2] In 1943 Adlersberg and Fox[3] reporting the results of a water tolerance test as one measure of liver function in dogs and humans noted a distinct decrease in the rate of urine excretion in both when the liver was damaged. Several observers[4,5] have reported a decrease in the excretion of urine during water tolerance tests in rats fed high-fat diets. This type of diet is associated with fatty infiltration of the liver and, if continued for a long period, with fibrosis.

In the present study water tolerance tests were carried out in patients with clearly established cirrhosis of the liver at periods when ascites was rapidly reaccumulating and at periods when the patients were free of ascites. Determinations of several of the serum constituents were also made during the course of the tests.

PROCEDURE

The tests were done in the fasting state. The patient was catheterized and 1,500 ml. of tap water were then administered orally. In order to obviate the factor of absorption several subjects were given 1,000 ml. of 5 per cent glucose intravenously. The rate of urine flow was accurately determined by collecting the urine by catheter at thirty-minute intervals. The first urine specimen was taken thirty minutes after the onset of hydration. Specific gravity and chloride concentration were determined in each urine sample. In all tests fasting blood samples were collected and other samples were taken at periods during the test which coincided with marked changes in the rate of urine excretion. All blood samples were drawn under oil. Plasma volume determinations using T-1824[6] were made in subjects in the basal state on the day prior to the tests. Total protein, albumin and globulin were determined by the method of Howe.[7] Serum chlorides were estimated by a modification of Sendroy's method.[8] Serum sodiums were determined by the method of Butler and Tuthill as described by Peters and Van Slyke.[9] Serum water determinations were made by the method described by Eisenman, MacKenzie and Peters.[10]

A summary of the information on the patients at the time they were admitted to the hospital is given in Table I. Their nutritional state varied from poor to good. Clinical evidence of ascites was present in all but two of the patients but in seven patients paracenteses were not necessary and the ascites disappeared during the course of therapy. A total of thirty water tolerance tests were done. The normal subjects are referred to as group I. The results in the fifteen patients with cirrhosis are divided into two groups, depending on whether or not ascites was prominent in the patients at the time of the test. Group II included patients who were free of ascites or in whom the ascites was minimal at

* From the Department of Medicine, New York University College of Medicine and The Third (New York University) Medical Division, Bellevue Hospital, New York, N. Y. This research was aided by a Grant from the United States Public Health Service.

† Now at Veterans Administration Hospital, White River Junction, Vt.

the time of the test. Group III consisted of patients who were rapidly reaccumulating ascitic fluid at the time of the tests. Repeated tests were made during the course of the disease in five of the patients and therefore the data on these patients are included in both groups II and III.

TABLE I
DATA ON PATIENTS WITH CIRRHOSIS AND ON NORMAL SUBJECTS

Case No.	Age (yr.)	Sex	Nutritional Status	Ascites	No. of Paracenteses	Serum Protein on Admission (gm. %)		
						Total	Albumin	Globulin
33C	43	M	Good	None	None	5.4	2.4	3.0
44C	40	M	Good	Yes	None	5.7	1.8	3.9
1	49	M	Poor	Yes	8	6.5	2.5	4.0
4	46	M	Poor	Yes	5	6.2	2.6	3.6
8	53	M	Obese	Yes	None	6.7	3.4	3.3
12	45	M	Fair	Yes	None	6.7	3.4	3.3
23	42	M	Obese	Yes	None	7.5	2.7	4.8
28	46	M	Poor	Yes	14	5.0	2.5	2.5
33	57	M	Fair	Yes	13	5.3	1.5	3.8
34	43	M	Good	Yes	12	7.8	2.1	5.7
48	42	M	Poor	None	None	8.1	2.7	5.4
49	33	M	Fair	Yes	None	6.8	2.4	4.4
61	42	M	Poor	Yes	13	4.9	2.2	2.7
69	67	M	Poor	Yes	14	7.6	2.7	4.9
70	59	M	Fair	Yes	None	5.2	2.0	3.2
Normals								
Sp	33	M	Thin	....	..	5.7	3.7	2.0
Le	26	M	Good	....	..	7.3	4.2	3.1
Ge	23	M	Thin	....	..	6.9	...	...
Cl	25	M	Good	....	..	6.4	4.5	1.9
Ov	27	M	Good	....	..	7.2	4.8	2.4
Co	21	M	Good	....	..	6.8	...	...
Li	38	M	Good	....	..	7.0	4.6	2.4

RESULTS

Effects of Water Tolerance Tests on Total Urine Output, Minute Volume, Chloride Excretion and Specific Gravity. The water tolerance tests are reported as the per cent of ingested water excreted at intervals of thirty minutes for a period of 240 minutes. The averages of the results are shown in Figure 1 for all three groups along with the averages of the minute volumes. The averages of the chloride excretion and of the specific gravity of the urines are given in Table II. In the normal subjects the average time required for excretion of 50 per cent of the ingested water was ninety-two minutes, and 100 per cent excretion was reached at 184 minutes. The average maximum minute volume was 11.6 ml./minute and occurred between 60 and 120 minutes. At the height of the diuresis the specific gravity of the urine ranged from 1.001 to 1.003.

In group II, cirrhosis patients without ascites at the time of the test (Cases No. 33C, 44C, 1, 4, 8, 12, 23, 28, 34, 48, 49, 61), the maximum urine flow also occurred between 60 and 120 minutes after the onset of hydration and reached an average of 11.1 ml./minute. The average of the specific gravity of the urine was higher (1.015) at thirty minutes than in the normal subjects but at the height of diuresis it had decreased to an average of 1.004. In these respects the average response to the ingested water was similar to that obtained in the normals (Fig. 1) but, unlike the normal subjects, a sharper drop in minute volume occurred at 120 minutes after the peak of diuresis; 50 per cent excretion of the ingested water did not occur until 100 minutes after the onset of hydration and 100 per cent excretion was not attained by the end of the experimental period (240 minutes).

In group III (patients with reaccumulating ascites, Cases No. 1, 33, 34, 69, 70), the maximum minute volume of urine flow was reached after eighty-five minutes and the average was only 4.2 ml./minute. The minute volume never exceeded 9 ml./minute. Fifty per cent excretion of the ingested water was not achieved during the experimental period except in two instances and in these cases was reached at 241 minutes. In the patients with ascites the volume of urine excreted at thirty minutes was insufficient for specific gravity determinations in all but two instances. At the peak of diuresis the average specific gravity was 1.004 in spite of a diminished urine volume. By the end of the test the specific gravity had risen to an average of 1.017, which was higher than in the other two groups.

The graphic representation of the minute volume (Fig. 1) illustrates the severe depression in the minute volume in the patients with ascites and also the rather sharp decrease in minute volume occurring at 120 minutes in the cirrhosis patients without

ascites. These are in marked contrast to the findings in normal subjects.

The excretion of chloride was significantly lower throughout the tests in the patients with severe ascites (group III). This is not explained by the chloride intake as the patients in both groups II and III were not on salt-free or salt-poor diets. The patients without ascites excreted approximately the same amount of chloride during the test as did the normal subjects. Farnsworth[11] also observed an almost complete absence of sodium in the urine of patients with cirrhosis of the liver. The analyses in her studies were done on urines collected during two clearance periods of two hours each.

TABLE II

CHLORIDE EXCRETION AND SPECIFIC GRAVITY OF URINE DURING THE WATER TOLERANCE TESTS

Groups	Urine Analyses	Time in Minutes							
		30	60	90	120	150	180	210	240
I	Specific gravity	1.011	1.005	1.002	1.003	1.004	1.006	1.008	1.007
	Cl, mEq./min.	.24	.19	.20	.20	.16	.18	.26	.19
II	Specific Gravity	1.015	1.008	1.004	1.003	1.005	1.007	1.008	1.011
	Cl, mEq./min.	.19	.19	.19	.18	.17	.15	.15	.17
III	Specific Gravity	.....	1.008	1.004	1.006	1.008	1.007	1.009	1.017
	Cl, mEq./min.	.02	.03	.04	.04	.04	.02	.02	.02

Effect of Intravenous Water Tolerance Tests. The results of administering 1,000 ml. of 5 per cent glucose intravenously to three subjects are shown in Table III. One subject was a normal (Li), one a patient free of ascites (Case No. 28) and one a patient with rapidly reaccumulating ascites at the time of the test (Case No. 1). The normal subject excreted 100 per cent of the infused glucose in eighty-seven minutes. The patient in whom ascites was not present excreted 100 per cent in 112 minutes. In the patient with cirrhosis and ascites only 26 per cent of the administered water had been excreted after 143 minutes. This table illustrates again the differences in chloride excretion in patients with and without ascites. The amounts of chloride excreted were very low in the patients with cirrhosis and ascites. Oral water tolerance tests had been made in these three subjects several days before the intravenous test. The normal subject excreted urine at the usual rate, i. e., 50 per cent of the ingested water was excreted after eighty-eight minutes. Case No. 28 (no ascites) required 193 minutes to excrete 50 per cent of the ingested water. To demonstrate the comparative effects of the oral and intravenous tests in Case No. 1 (with severe ascites), the results are shown graphically in Figure 2. It is clear that in both tests the excretion of urine was significantly depressed. It seems from these observations that in patients with cirrhosis of the liver, with or without ascites, the administration of fluid, either intravenously or orally, is associated with a depression in urine flow.

The blood sugar levels were also determined in the intravenous tests, as the administered fluid contained 5 per cent glucose. A rapid fall in the blood sugar occurred in the normal subject while in the patients with cirrhosis the blood sugar rose above normal level and fell more slowly. (Table III.)

Effect of Intra-abdominal Pressure on the Water Tolerance Test. Because of the effect of the hydrostatic pressure imposed by the accumulation of fluid in the abdomen, the tests on the patients with ascites were made soon after paracentesis, at which time the abdomen was not distended with fluid. The

effect of the hydrostatic pressure of the ascitic fluid is illustrated in Case 33. (Fig. 3.) One test was made immediately after a paracentesis and a second test when the abdomen was greatly distended with fluid. At the time of these tests there were no

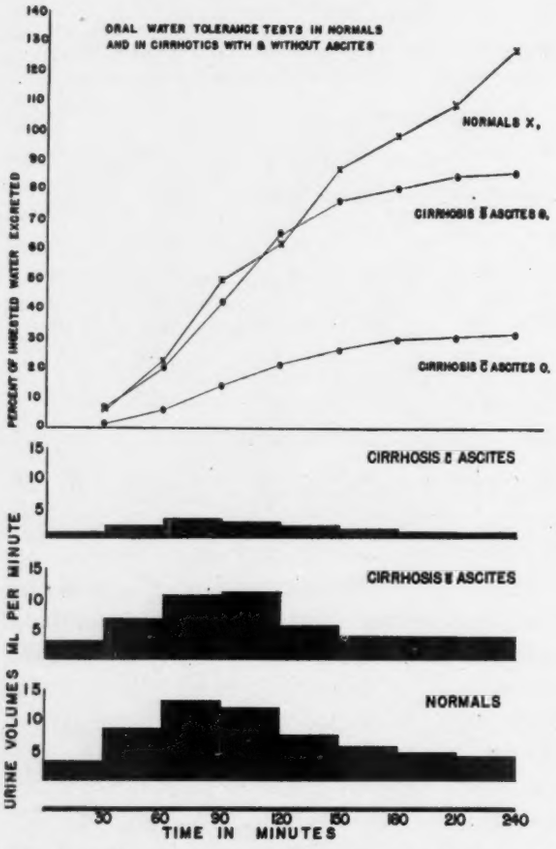
ORAL WATER TOLERANCE TESTS IN NORMALS AND IN CIRRHOTICS WITH & WITHOUT ASCITES
PERCENT OF INGESTED WATER EXCRETED
NORMALS X,
CIRRHOSIS ♀ ASCITES ⊕,
CIRRHOSIS ♂ ASCITES ⊖,
URINE VOLUMES ML PER MINUTE
CIRRHOSIS ♀ ASCITES
CIRRHOSIS ♂ ASCITES
NORMALS
TIME IN MINUTES

FIG. 1. Averages of water tolerance tests and of minute volume of urine during the tests in normal subjects and in patients with cirrhosis of the liver with and without ascites.

differences in the serum levels of total protein, albumin, globulin or chloride. The excretion of urine was significantly depressed during both of the water tolerance tests; but when the abdomen was distended with fluid, only 7 per cent of the ingested water was excreted during the period of the test in contrast to 18 per cent excretion when the ascitic fluid had been removed.

Repeated Water Tolerance Tests during the Course of Therapy. The patients received intensive therapy consisting of a highly nutritious diet, vitamins and, in the patients with severe ascites, large amounts of a specially prepared liver extract which was given intravenously.[12] As a result of this combined method of therapy all but two of the patients with ascites (Cases 33 and 70) eventually ceased to reaccumulate ascitic fluid. Repeated water tolerance tests were

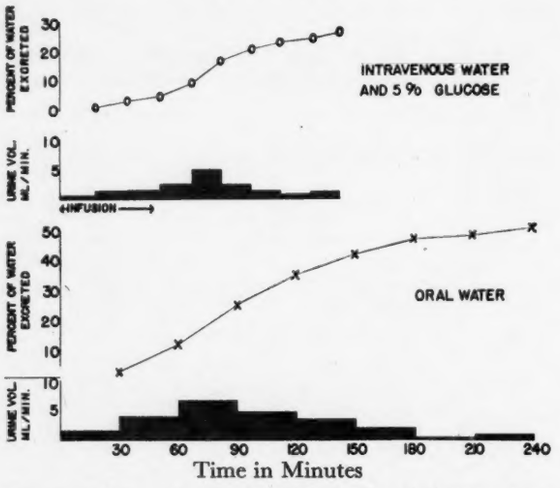

FIG. 2. Effects of oral administration of 1,500 ml. of water and of intravenous administration of 1,000 ml. of 5 per cent glucose in Case 1, a patient with cirrhosis of the liver and ascites at the time of the tests.

made during the course of therapy and invariably, once ascites was controlled, the per cent of the ingested water excreted increased. Figure 4 illustrates the results of repeated water tolerance tests in Case 34, a forty-six year old male with severe liver damage and ascites. Over a five-year period this patient had had six admissions to the hospital. During each admission he received intensive therapy. In the early phase of the disease his ascites subsided by spontaneous diuresis. The water tolerance test at this time (May 2, 1946) is represented by the crosses and is abnormal. Eight months later he returned to the hospital and at this time required ten paracenteses over a period of nine months, with the removal of a total of 50 L. of ascitic fluid. An oral water tolerance test performed at the time ascites was severe was associated with a marked depression of urine output (January 14, 1947, solid circles). Intensive therapy, with diet and liver extract given intravenously, eventually controlled the ascites and another water tolerance test was done two months

TABLE III

EFFECT OF 5% GLUCOSE IN WATER (1,000 ML.), GIVEN INTRAVENOUSLY, ON URINE OUTPUT IN PATIENTS WITH CIRRHOSIS, WITH AND WITHOUT ASCITES, AND IN A NORMAL SUBJECT

	Elapsed Time (min.)	Urine Analyses					Blood Analyses	
		Urine Volume (ml.)	Minute Volume (ml.)	Per cent Ingested H_2O Excreted	Specific gravity	Chloride (mEq./min.)	Blood Sugar (mg. %)	Serum Chloride (mEq./L.)
Case 1 Cirrhosis with Ascites	0						72	98
	18	12	0.7	1.2	1.018	0.12		
	35	17	1.0	2.9	1.020	0.30		
	50*							
	51	18	1.1	4.7	1.020	0.16		
	54						201	92
	68	45	2.6	9.2	1.011	0.08		
	83	75	5.0	16.7	1.006	0.15		
	90						146	101
	98	37	2.5	20.4	1.002	0.05		
	113	22	1.5	22.6	1.008	0.04		
	128	13	0.8	23.8	1.010	0.02		
	143	20	1.3	25.8	1.006	0.03		
	144						78	99
Case 28 Cirrhosis, No Ascites	0						84	99
	17	27	1.8	2.7	1.022	0.39		
	32	86	5.7	11.3	1.012	0.62		
	40*							
	42						238	99
	47	170	11.3	28.3	1.005	0.37		
	64	225	13.2	50.8	1.003	0.20		
	78	173	12.3	68.1	1.001	0.10		
	91	166	12.8	84.7	1.003	0.15		
	94						125	101
	106	121	8.1	96.8	1.002	0.11		
	121	86	5.7	105.4	1.002	0.20		
	137	58	3.6	111.2	1.005	0.08		
	138						80	101
Case LI Normal Subject	0						96	102
	16	75	4.7	7.5	1.007	0.40		
	33	156	9.2	23.1	1.005	0.43		
	45*	158	13.1	38.9	1.004	0.41		
	50						187	100
	60	233	15.5	62.2	1.003	0.46		
	75	222	14.8	84.4	1.003	0.34		
	90	198	13.2	104.2	1.002	0.39		
	93						51	100
	106	107	6.7	114.9	1.007	0.42		
	120	114	8.1	126.3	1.007	0.51		
	133						57	101
	137	126	7.4	138.9	1.008	0.23		

* Infusion completed.

after the last paracentesis (October 28, 1947), at which time he was entirely free of ascites. The rate of urine excretion with this test was unusually high; 50 per cent of the ingested water was excreted in 67 minutes and 100 per cent in 109 minutes. The

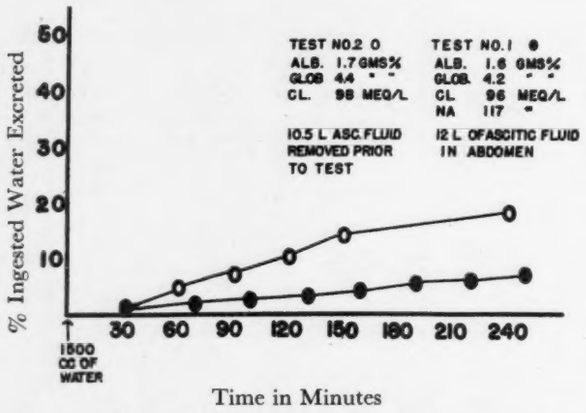

FIG. 3. Effect of ascitic fluid in the abdomen on the water tolerance test in Case 33. Test No. 1 was done when the abdomen was distended with ascitic fluid. The actual amount (12 L.) was determined by paracenteses after the test. Test No. 2 was done immediately after the removal of 10.5 L. of fluid from the abdomen. Both tests show a greatly decreased urine output following the administration of 1,500 ml. of water orally. When ascites was present the depression was greatest.

minute volume at the peak of diuresis reached 20.1 ml. per minute. The results illustrate the improvement in water tolerance tests which corresponded with control of the reaccumulation of ascitic fluid.

Determinations of the Serum Constituents in Patients with Cirrhosis and the Changes in Serum Constituents Occurring during the Water Tolerance Tests in Normal Subjects and in Patients with Cirrhosis. During this study serum determinations in the fasting state were made in a large group of patients with cirrhosis, with and without ascites. The averages of these results are given in Table IV. The serum water is expressed as gm./kg. of serum. The other serum constituents are reported as gm. per cent or mEq./L. and also as gm. or m-osM/kg. of serum water. The latter method of reporting the data was used in order better to compare the changes in the osmotically active serum constituents during the experiments. As a result of these analyses two situations were observed in the patients with severe ascites (group III) and this group is subdivided to illustrate these differences. In group III-A, consisting of four patients, the serum water was elevated, ranging from 940 to 945

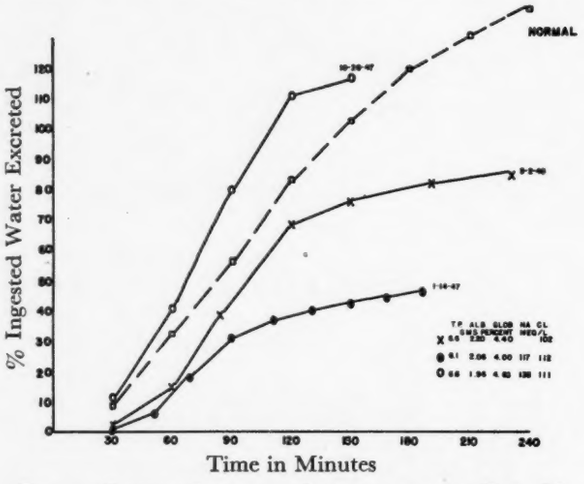

FIG. 4. Repeated water tolerance tests in Case 34 during the course of the disease. In May, 1946, early in the disease, ascites subsided spontaneously and the water tolerance test was depressed. In January, 1947, the ascites required repeated paracenteses and the water tolerance test was still more depressed. As a result of therapy the ascites was controlled. The test in October, 1947, was made after ascites had been controlled and the patient excreted the ingested water more rapidly than did normal subjects. The broken line represents the results in a normal subject for comparison.

gm./kg. of serum and the serum albumin was very low, averaging 22 gm./kg. of serum water. In the patients in group III-B, who also required frequent and repeated paracenteses, the serum water was lower, ranging from 932 to 921 gm./kg. of serum and the albumin was higher, averaging 33 gm./kg. of serum water. Clinically the patients in group III-A were poorly nourished and did not respond well to therapy. Two of these patients have died, one is still under treatment and in one patient ascites was finally controlled. During the time when the latter patient had marked ascites the serum water was 940 gm./kg. of serum; once ascites was controlled the serum water fell to 935 gm./kg. of serum. This was associated with an increase in serum albumin from 27 to 34 gm./kg. of serum water. Ap-

parently increased serum water with associated decrease in serum proteins, particularly in the albumin fraction, indicates a more severe situation clinically and is probably evidence of a profound depletion of tissue protein.

TABLE IV

AVERAGES OF THIRTY-FOUR FASTING BLOOD SERUM ANALYSES IN PATIENTS WITH CIRRHOSIS OF THE LIVER

Groups	Total Protein (gm. %)	Albumin (gm. %)	Globulin (gm. %)	Sodium (mEq./L.)	Chloride (mEq./L.)	Serum Water (gm./kg. Serum)	Proteins (gm./kg. Serum H_2O)			Sodium (m-osM/kg. Serum H_2O)	Chloride (m-osM/kg. Serum H_2O)
							Total	Albumin	Globulin		
I Normals	6.4	4.6	1.8	137	103	936.0	68.4	49.0	19.4	145.8	109.9
II Cirrhosis without Ascites	6.9	4.0	2.9	138	103	926.7	73.8	42.4	31.4	146.4	110.9
III Cirrhosis (A) with Ascites* (B)	5.4	2.2	3.4	136	106	944.6	57.1	21.9	35.2	144.5	113.0
	7.0	2.9	4.0	135	103	927.7	75.3	32.9	42.9	145.0	111.4

* Patients with rapidly reaccumulating ascites; in the patients in Group III-A the serum water was elevated and the total proteins were lower than in the patients in III-B.

The patients in group II had no demonstrable ascites at the time of the analyses. This group, however, consisted of patients in whom ascites had been controlled as a result of therapy and of patients in whom ascites was never severe enough to require paracentesis. We found that once ascites was controlled, there were no significant differences in serum water, proteins, sodium and chloride in the two types of patients included in this group.

The experiments reported in Table v were made in patients selected as typical of the larger group of patients. As stated before, twenty-nine tests in all were carried out. Blood samples for analyses were taken immediately before the administration of water, at or near the time of maximum urine flow and at the end of the experiment.

In the normal subject the minute volume of urine reached a peak of 15.5 ml., ninety-six minutes after the water was administered. By this time 52 per cent of the ingested water was excreted. The blood sample taken within nine minutes showed an increase of 3.0 per cent in the total serum proteins and this was due to an increase in serum albumin. The sodium chloride had decreased 2 per cent from the fasting level. The minute volume of urine had decreased to 3.8 ml. at 185 minutes and by this time 99 per cent of the ingested water had been excreted. A blood sample taken at 210 minutes showed a decrease in serum proteins of 7 per cent from the level reached at the peak of diuresis and an increase in the sodium chloride of 2.8 per cent from the level obtained during the height of diuresis. Five minutes after this blood sample the minute volume of urine had decreased to 2.2 ml.

Case No. 4 was a patient with cirrhosis of the liver who was actively diuresing at the time of the test. The diuresis had begun four days before the test and was associated with a decrease of ascites so that the patient required no further paracentesis. Eighty-nine minutes after hydration the minute volume was 14.0 ml., the total serum proteins had increased 2 per cent and this increase was in the globulin fraction in contrast to the findings in the normal subject. The sodium chloride had decreased 4.5 per cent from the fasting level; one hundred per cent of the ingested water was excreted after 172 minutes and at this time the minute volume was 3.1 ml. The serum analyses corresponding to the decrease in the minute volume of urine showed a decrease in the total proteins of 2.5 per cent from the level obtaining at the peak of diuresis and the globulin fraction had returned to the fasting level. The sodium

chloride had increased 2.8 per cent from the level reached during maximal diuresis.

Case No. 28 was a patient who had required fourteen paracenteses over a period of eleven months. The last tap had been done fifteen weeks before this water tolerance test and there was no evidence of ascitic fluid at this time. The peak of diuresis was 12.1 ml. and did not occur until 120 minutes after hydration. The blood sample was taken at ninety-three minutes, which was during the period when the minute volume of urine was increasing rapidly. At this time the serum proteins had increased 2.8 per cent and, as in Case No. 4, the increase was in the globulin fraction. The sodium chloride had decreased 3.6 per cent from the fasting level. This patient had excreted only 70 per cent of the ingested water by the end of the test, at which time

TABLE V

CHANGES IN SERUM CONSTITUENTS DURING WATER TOLERANCE TEST IN A NORMAL SUBJECT AND THREE PATIENTS WITH CIRRHOSIS OF THE LIVER

	Elapsed Time (min.)	Urine Determinations				Serum Analyses											Per cent Change in NaCl
		Minute Volume (ml.)	Specific Gravity	Per cent Excretion	Chloride (mEq./min.)	Protein (gm. %)			Sodium (mEq./L.)	Chloride (mEq./L.)	Serum Water (gm./kg.)	Protein (gm./kg. Serum H_2O)			Sodium (m-osM/kg. Serum H_2O)	Chloride (m-osM)/kg. Serum H_2O	Sum of Sodium and Chloride	
						Total	Albumin	Globulin				Total	Albumin	Globulin				
Normal Subject Case "Cl"	45	2.4	1.006	7.3	0.29	6.4	4.6	1.8	137	103	936	68.8	48.8	20.0	146	110	256	
	65	9.3	1.004	19.8	0.18													−2.0
	96	15.5	1.001	51.7	0.25													
	105					6.7	4.8	1.9	135	100	937	71.4	51.2	20.2	144	107	251	
	126	13.5	1.003	78.9	0.24													
	155	6.5	1.004	91.3	0.22													
	185	3.8	1.007	99.0	0.28													
	210					6.2	4.5	1.7	138	103	936	66.2	48.1	18.1	148	110	258	+2.8
	215	2.2	1.010	103.3	0.25													
Case 4 Tap 14 days before test; diuresis had begun 4 da. before test	0					7.0	2.9	4.1	139	107	929	75.0	31.2	43.8	150	115	265	
	15																	
	30	4.4	1.011	8.8	0.49													−4.5
	61	11.1	1.003	31.6	0.32													
	89	14.0	1.001	57.7	0.23													
	92					7.1	2.9	4.2	133	102	931	76.6	31.1	45.5	143	110	253	
	120	13.5	1.001	85.8	0.22													
	149	5.4	1.003	96.4	0.32													
	178	2.3	1.007	101.0	0.16													+2.8
	208	3.1	1.008	107.3	0.14													
	211					7.0	2.9	4.1	135	107	934	74.7	31.0	43.7	145	115	260	
Case 28 Ascites controlled: last tap 15 wk. before test	0					6.4	4.4	2.0	135	100	932	68.5	47.2	21.3	145	108	253	
	34	0.9	1.023	2.0	0.21													
	61	0.4	1.023	2.8	0.08													
	90	6.6	1.007	12.8	0.34													−3.6
	93					6.6	4.0	2.6	129	99	935	70.4	42.8	27.6	138	106	244	
	120	12.1	1.002	37.2	0.25													
	152	3.6	1.003	44.8	0.13													
	186	2.1	1.008	48.7	0.13													
	210	4.4	1.006	56.5	0.15													
	233					6.4	3.9	2.5	135	102	935	68.2	41.7	26.5	144	109	253	+3.7
	235	8.3	1.003	70.4	0.33													
Case 1 Ascites present: tap 4 days before test	0					6.6	2.4	4.2	133	105	928	71.4	25.9	45.5	143	113	256	
	30	0.7	1.020	1.3	0.07													
	60	2.8	1.008	6.9	0.06													−3.5
	90	6.6	1.006	20.1	0.06	7.2	2.8	4.4	129	100	929	77.5	30.2	47.3	139	108	247	
	120	7.3	1.004	34.8	0.10													
	150	6.1	1.004	46.8	0.08													
	179	5.2	1.004	57.1	0.10													+4.5
	211	2.8	1.005	63.0	0.06													
	238					7.2	2.8	4.4	136	104	928	77.5	30.2	47.3	146	112	258	
	240	1.5	1.008	66.0	0.04													

the serum proteins had decreased to the fasting level but the albumin fraction was still below the fasting level. By the end of the test the sodium chloride had increased 3.7 per cent from the level obtained at ninety-three minutes.

tained ninety minutes after the administration of water.

In order to duplicate in a normal subject the situation occurring during a water tolerance test in patients with cirrhosis, pitressin was injected subcutaneously into

TABLE VI

EFFECT OF INJECTION OF PITRESSIN IN A NORMAL SUBJECT AFTER ADMINISTERING 1,500 ML. OF WATER ORALLY

Urine Analyses							Serum Analyses											
Time from Completion of Hydration (min.)	Total Urine Volume (ml.)	Minute Volume Urine (ml.)	Specific Gravity	Per cent Excretion	Urine Na (mEq./min.)	Urine Cl (mEq./min.)	Proteins (gm. %)			Na	Cl	Serum H_2O (gm./kg. Serum)	Proteins (gm./kilo. Serum H_2O)			Na and Cl (m-osM/kg. Serum H_2O)		
							Total	Albumin	Globulin	(mEq./L.)			Total	Albumin	Globulin	Na	Cl	Sum of NaCl
0 (Hydration)	0						7.2	4.8	2.4	138	105	930	78	52	26	148	113	261
60	330	5.7	1.004	22	.09	.15												
90	370	12.3	1.002	47	.04	.10												
93							6.8	4.9	1.8	134	102	931	73	53	20	144	110	254
98	8 pressor units of pitressin given subcutaneously																	
139	0																	
145							6.7	4.9	1.8	133	103	933	72	53	19	143	111	254
157	185	2.8	1.006	59	.06	.10												
184							6.9	5.0	1.9	134	105	931	74	54	20	144	113	257
197	31	0.8		61	.06	.05												
222	11	0.4		62	.04	.03												
238							6.8	4.7	2.1	131	105	930	73	51	22	141	113	254
239	9	0.5		63	.04	.07												

Case No. 1 was a patient requiring frequent paracenteses, the last four days before the test. Ninety minutes after the administration of water the minute volume was 6.6 ml., the total serum proteins had increased 8.5 per cent and in this case both the albumin and globulin fractions rose. The sodium chloride had decreased 3.5 per cent from the fasting level. The minute volume reached a peak of 7.3 ml. thirty minutes later and thereafter diminished so that only 66.0 per cent of the ingested water was excreted in 240 minutes. At this time the total serum proteins had not changed from the level obtained at ninety minutes. The sodium chloride level, however, had increased 4.5 per cent from the level ob-

a normal subject at the time the minute volume had almost reached its peak. The results of this experiment are shown in Table VI. Ninety minutes after hydration the minute volume was 12.3 ml. and 47 per cent of the ingested water had been excreted. The usual decrease had occurred in the serum sodium and chloride. Eight pressor units of commercial pitressin were given subcutaneously. In the fifty-nine minutes following pitressin the minute volume decreased to 2.8 ml. per minute, and for the rest of the test it ranged from 0.8 to 0.4 ml. per minute. Blood taken eighty-six minutes after pitressin showed an increase in sodium chloride of 1 per cent. The milliequivalents of chloride excreted

per minute decreased during the period of severe antidiuresis. If these results are compared with those obtained in the patients with cirrhosis in Table v, the antidiuretic effect after pitressin is seen to be even greater than was observed in the patients with cirrhosis. However, the changes in serum sodium and chloride did not accompany antidiuresis resulting from pitressin.

TABLE VII
ASCITIC FLUID AND SERUM ANALYSES DURING THE COURSE OF THE WATER TOLERANCE TEST

Case No.	Time after Hydration (min.)	Ascitic Fluid Determination			Serum Analyses			
		Total Protein (gm. %)	Na (mEq./L.)	Cl (mEq./L.)	Total Protein (gm. %)	Na (mEq./L.)	Cl (mEq./L.)	Per cent of Ingested Water Excreted
33	0	0.58	131	109	5.8	117	96	
	130	0.60	133	107	6.2	114	95	3.5
	248	0.58	123	106	6.5	119	95	7.0
34	0	0.56	132	120	6.1	117	112	
	199	1.25	130	86	5.8	123	119	46
70	0	1.06	142	112	6.1	137	108	
	107	1.12	137	112	6.0	137	105	33
	195	0.95	139	113	6.2	139	104	48
69	0	1.49	138	112	8.1	136	106	
	97	1.67	139	115	8.0	142	107	19
	194	1.44	137	113	7.9	136	109	37

Ascitic Fluid Determinations during Water Tolerance Tests. In four cases (No. 33, 34, 69 and 70) samples of ascitic fluid were withdrawn during the tests and were analyzed for total proteins, sodium and chloride. (Table VII.) Cases 33 and 34 were patients in whom cirrhosis of the liver had been present for many months and whose ascites had existed for a long period of time. In both these patients the total protein in the ascitic fluid was low. Cases 69 and 70 were patients with severe cirrhosis but the ascites had been present for a shorter period of time and the ascitic fluid proteins were higher. Water was the only substance administered for the test and as this was given orally it was probably added only gradually to the body fluids. The changes occurring in the ascitic fluid, although not striking, are suggestive and it would appear from the results that at the height of diuresis an alteration had occurred in the distribution of Na and Cl between the ascitic fluid and the serum. At the end of the experiment, when urine flow was diminished, these changes tended to reverse themselves.

TABLE VIII
PLASMA VOLUME DETERMINATIONS

	Average (cc.)	Range (cc.)
Group I Normal subjects	2,998	2,620–3,250
Group II Cirrhosis patients without ascites	3,600	3,290–3,960
Group III Cirrhosis patients with ascites	.....	.....
(A) During period of rapid reaccumulation of ascites	5,400	4,420–6,730
(B) During period of diminishing ascites under therapy	4,680	4,000–5,250

Plasma Volume Determinations. Plasma volume determinations, using the dye T-1824, were made in all patients. In patients with ascites the determinations were carried out both during the period of ascitic fluid reaccumulation and after ascites was controlled. In all of the patients with cirrhosis, regardless of whether or not ascites was present, the plasma volumes exceeded the range of the normal controls. It was also found that the greatest increase in plasma volume occurred in patients during periods when ascites was rapidly reaccumulating. The results are shown in Table VIII and the patients with ascites are subdivided according to whether ascites was reaccumulating rapidly or whether ascites was diminishing. Similar observations of an increased plasma volume have been reported by Perera[13] in patients with cirrhosis of the liver. Labby et al.[14] found an increase in plasma volume in patients with infectious hepatitis.

COMMENT

The occurrence of oliguria in patients with cirrhosis of the liver has long been accepted as a classic manifestation of the disease.[1] The results of the water tolerance

tests reported in these experiments demonstrate a decreased ability to excrete ingested water in patients with cirrhosis, most marked when ascites is associated with the disease. When recovery took place as a result of therapy, the water tolerance test improved and in several instances the patients excreted the ingested water at a more rapid rate than did normal subjects. In the patients with ascites the tests were made at intervals between paracenteses in order to evaluate the effect of the hydrostatic pressure exerted by the ascitic fluid. Even when the tests were made immediately following paracentesis so that no fluid was present in the abdominal cavity, the water tolerance test was consistently and significantly depressed. Other observers have reported decreased water tolerance tests in patients with chronic liver disease[3] and Labby and Hoagland[14] observed it in patients with infectious hepatitis in whom ascites was never a complication.

We have suggested before[15] that the decreased urine output in patients with cirrhosis of the liver was a factor contributing to the production of ascites. It has been repeatedly observed that when the kidney can increase the rate at which it excretes water, thus establishing a diuresis, ascites will subside.[12,15] The effect of diuresis in overcoming ascites has been the basis of the use of mercurial diuretics in patients with cirrhosis of the liver.[16] What has not been established is the sequence of events leading to fluid retention in patients with liver disease. One factor which could contribute to a decreased urine output would be an increase in the amount of the antidiuretic hormone of the posterior pituitary. An increase of this hormone could be the result either of decreased inactivation[17] by the damaged liver or it could be due to an increased secretion of the hormone. There is adequate experimental evidence as a result of Verney's work[18,19] that stimulation to the secretion of the antidiuretic hormone is through the osmoreceptors of the supraopticohypophyseal tract. These receptors are situated in the bed of the internal carotid artery and changes in the osmotic pressure of the carotid blood stimulate the osmoreceptors, thus causing a discharge of the antidiuretic hormone. Liberation of the antidiuretic hormone is continuously governed by the concentration of sodium chloride and of other osmotically active substances in the arterial plasma. Verney reported that an increase of only 2 per cent in the osmotic pressure of the carotid blood "reduced the urine flow during water diuresis to about 10% of its maximum." The response was almost entirely abolished after removal of the posterior lobe of the pituitary. The effect of hypertonic saline in stimulating the posterior pituitary has been used clinically as a diagnostic procedure in diabetes insipidus.[20]

In the experiments that we have reported the most striking changes in the serum constituents occurring during the water tolerance tests were in the concentrations of sodium chloride. During the period of diuresis following the administration of fluid the sodium chloride concentration in the serum decreased; during the period when the minute volume of urine was decreasing the concentration of these electrolytes increased. The fluctuations were greater in patients with cirrhosis than in normal subjects. In the normals the decrease of NaCl at the peak of diuresis averaged 2 per cent and the increase in NaCl, when the minute volume of urine was diminishing, averaged 2.5 per cent. In patients with cirrhosis the average NaCl decrease at the times of maximum urine flow was 4 per cent and the average increase in the concentration of NaCl, above the level obtaining at the peak of diuresis, was 3.5 per cent. It would seem that an increase of such magnitude could serve to stimulate secretion of the antidiuretic hormone and that this would account for the sharp decrease in the minute volume of urine. The changes in the concentrations of NaCl observed in these patients would fit in with Verney's hypothesis[18] and would explain the low urine output that occurs in patients with cirrhosis of the liver.

Cirrhosis of the liver is a disease which has widespread metabolic effects and we do not mean to infer that the depressed urine volume is the sole explanation of the disturbance in water balance occurring in these patients. However, it may well be that the predominance of ascites in this disease is due to the combination of increased portal pressure, increased capillary permeability and water retention associated with the decreased urine output. If the disease is not too advanced, spontaneous diuresis may be brought about by various therapeutic measures such as a highly nutritious diet, intensive therapy with vitamins, mercurial diuretics or a low-sodium diet. Once the disease has advanced so that ascites requiring repeated paracenteses has occurred, the production of diuresis is more difficult. In a certain number of cases this can be accomplished by the persistent use of proper diet, and we have found that the administration of large amounts of liver extract intravenously is also advantageous.[12] As reported previously[12] liver extract contains many growth-promoting factors and it has seemed to us that the stimulus to tissue regeneration is increased by this method of therapy. In our experience during the past ten years patients who have had as many as thirty-seven paracenteses have finally ceased reaccumulating ascites.

SUMMARY

Water tolerance tests were made in normal subjects and in patients with cirrhosis of the liver, without and with ascites. The capacity of the patients with cirrhosis to excrete ingested water was diminished. Normal subjects excreted 100 per cent of the ingested water in 180 minutes. In patients with cirrhosis of the liver without ascites, 100 per cent excretion of the ingested water did not occur during the test and at 180 minutes 80 per cent of the water had been excreted. In the patients with cirrhosis and ascites the average amount of the ingested water excreted by the end of the test was only 32 per cent.

The minute volume of urine was depressed during the entire test in the patients with ascites. In the patients without ascites the minute volume decreased sharply once the peak of diuresis was reached. The decrease in the capacity of the patients with cirrhosis to excrete water was also observed following the intravenous administration of 5 per cent glucose.

The concentration of sodium chloride in the serum fluctuated during the tests and the changes were greater in the patients with cirrhosis than in the normal subjects. In the patients with cirrhosis there was an increase of 3 per cent or more in the serum level of NaCl at the time the minute volume of urine was decreasing. It is suggested that this increase in sodium chloride may have served to stimulate the osmoreceptors, causing a discharge of the antidiuretic hormone. It is, therefore, possible that an increased secretion of the antidiuretic hormone is partly responsible for the decreased urine output in patients with cirrhosis of the liver and that the inability to excrete water, by contributing to the retention of fluid, is a factor in the production of ascites.

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The Role of Shock in the Production of Central Liver Cell Necrosis*

MAX ELLENBERG, M.D. *and* KERMIT E. OSSERMAN, M.D.
New York, New York

In a study of hyperglycemia in coronary thrombosis[1] an association between hyperglycemia, shock and central liver cell necrosis became evident. The present study was undertaken to clarify the nature of the relationship of central liver cell necrosis to shock. The immediate problems to be investigated were: (1) What are the criteria for the pathologic diagnosis of central liver cell necrosis? (2) What is the relationship to shock? (3) What other clinical features are associated with its presence? (4) What is the role of congestive heart failure? To answer these problems a study of 200 routine unselected autopsy cases was made. The microscopic liver sections † of these cases were analyzed and then correlated with an independent study of the clinical history, which included the diagnosis, the presence of shock, duration and cause of shock, associated clinical phenomena and the presence or absence of congestive heart failure.

CRITERIA

It was evident early in our studies and from a survey of the literature that it would be necessary to define more clearly the criteria for the diagnosis of central liver cell necrosis. Many previous reports have not differentiated between necrosis, degeneration and severe degrees of atrophy, thus resulting in confusion and contradiction.

In the clear, well defined cases of central liver cell necrosis in our series the following features were present: (1) There was marked congestion of the central vein and marked distention of the sinusoids in the central area. Frequently there was local hemorrhage and extravasation of red blood cells, with rupture of the sinusoids. Not infrequently agglutination of the red blood cells was present. (2) Eosinophilic staining of the involved area was apparent, contrasting sharply with the basophilic stain of the normal liver cells. (3) Nuclear changes were present. These consisted of pyknosis, disintegration, fading nuclei and the occasional finding of nuclei lying free of the cytoplasm. (4) Polymorphonuclear leukocytic infiltration was always present although varying considerably in degree. (5) Architectural disruption attendant upon the necrosis was a constant finding but varied considerably in proportion to the extent of the necrotic process. Necrosis was almost invariably centrilobular in distribution, with the most advanced changes being present in the center and diminishing toward the periphery of the lobule. The line of demarcation between necrotic and normal tissue was usually well defined, with the surrounding cells in the normal area showing a ground-glass appearance of the cytoplasm. Infrequently, the necrosis was irregularly distributed rather than central in location.

Using these criteria we eliminated from our series of unequivocal cases what may be referred to as a border-line group. This group will be discussed later.

RELATIONSHIP OF SHOCK TO CENTRAL LIVER CELL NECROSIS

There were thirty-four instances of central liver cell necrosis in our series of 200 cases. Of these, thirty-two, or 94 per cent, were clearly associated with shock on the one hand and, conversely, were unassociated consistently with any other factor. The mere presence of shock, however, was not necessarily sufficient to result in central necrosis. An additional 61 of the 200 cases were also clearly associated with shock but

† These were all routine sections obtained at autopsy. We are aware of the possible objection that pathologic changes in the liver may vary from area to area. However, we believe that the large number of cases in this study minimizes the possible vagaries of chance representation.

* From the Laboratories of the Mount Sinai Hospital, Division of Pathology, and the Medical Services of the Mount Sinai Hospital, New York, N. Y.

failed to show the pathologic changes of central liver cell necrosis. Further analysis of these two groups disclosed a highly significant differentating factor, namely, the *duration* of shock. As seen in Table I, of the thirty-two patients with necrosis

TABLE I
DISTRIBUTION OF CASES IN RELATIONSHIP TO DURATION OF SHOCK

Duration of Shock (hr.)	No. of Cases Showing Central Liver Cell Necrosis	No. of Cases Not Showing Central Liver Cell Necrosis
0–10.....	2	41
10–24.....	5	18
24 or more..	25	2
	32	61

twenty-six had shock lasting at least twenty-four hours before death whereas of the sixty-one patients without necrosis all but two were in shock for less than twenty-four hours. Thus recognition of the importance of the time factor permits a correlation between the incidence of central liver cell necrosis and the presence of shock of some duration.

CLINICAL EVALUATION OF OUR CASES OF CENTRAL LIVER CELL NECROSIS

The clinical diagnoses, causes of shock, duration of shock, age and sex of the patients in our series who showed central liver cell necrosis at autopsy are listed in Table II. The absence of death with shock resulting from trauma or burns derives from the fact that our hospital has neither an ambulance nor a traumatic service; consequently, a high proportion of the cases represent so-called "medical shock." Thus nine cases resulted from acute coronary thrombosis, four from pulmonary embolization, four from acute heart failure, six from hemorrhage and the rest included sepsis, peritonitis, pleural aspiration, operation and acute pulmonary edema. Despite the wide range of factors precipitating the shock syndrome in our cases the pathologic findings fitted our criteria in every instance and were indistinguishable. As long as the time factor, i.e., the duration of shock, was about twenty-four hours or longer, central liver cell necrosis was present and the microscopic picture was of the same pattern. (Figs. 1 to 4.)

The clinical diagnoses associated with the cases of central liver cell necrosis again reflect

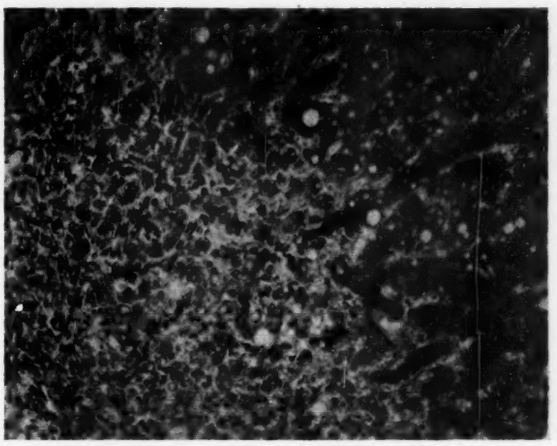

FIG. 1. Acute coronary thrombosis with shock. Note the sharp line of demarcation between necrotic and normal liver cells, leukocyte infiltration and congestion.

the high incidence of medical cases; nevertheless a wide variety of diagnoses are represented. These include malignancy, hepatic cirrhosis, hypertension, coronary disease, rheumatic fever, peptic ulcer and aneurysm. The relatively large number of cases with cardiac involvement will be considered separately.

CASES OF LIVER CELL NECROSIS WITHOUT SHOCK

There were two cases in our series of 200 that presented liver cell necrosis without manifest shock. Neither of these cases clearly fitted into the readily recognizable microscopic pattern of the other cases with shock. However, since necrosis was present it was believed that these cases should be included for purposes of statistical evaluation.

One of the two was a forty-three year old female with rheumatic chronic cardiovalvular disease who had been in failure for the last eight years of life. During the four days preceding death she developed a persistent ectopic tachycardia which varied from flutter to fibrillation with a pulse rate of 160 to 180 beats per minute, and an accompanying sharp decrease in cardiac output. Marked shock was not manifest. At autopsy the liver sections did not show central liver cell necrosis but there were areas of acute focal necrosis.

The second case was a fifty-nine year old male who had had an exploratory thoracotomy one year prior to his death, at which time an inoperable pulmonary carcinoma was found. He was admitted the second time because of bronchopneumonia. On the fourth day of

TABLE II

CLINICAL ASPECTS OF THE CASES WITH CENTRAL LIVER CELL NECROSIS

Age and Sex	Clinical Diagnosis	Associated Features	Duration of Shock	Cause of Shock	Congestive Heart Failure
58, M	Cirrhosis of liver	Diverticulitis	48 hr.	Hemorrhage	0
36, M	Malignant hypertension	Sympathectomy	12 hr.	Operation	0
47, M	Acute coronary thrombosis	Pulmonary embolization	48 hr.	Embolization	++
70, M	Carcinoma of penis	Operation, sepsis, purulent meningitis	3 days	Sepsis	0
45, M	Acute coronary thrombosis	Follicular lymphoblastoma	3 days	Coronary thrombosis	++++
70, F	Dissecting aortic aneurysm	Rupture into mediastinum	5 days	Hemorrhage	0
65, M	Acute coronary thrombosis	Renal calculi	24 hr.	Coronary thrombosis	0
63, F	Arteriosclerotic heart disease	Pulmonary embolization	36 hr.	Embolization	++
51, F	Carcinomatosis	Pulmonary embolization	36 hr.	Embolization	0
13, M	Rheumatic heart disease	Acute pulmonary edema, acute myocarditis	10 hr.	Acute pulmonary edema	++++
52, M	Rheumatic heart disease	Acute paroxysmal tachycardia	8 hr.	Coronary insufficiency	++++
52, M	Acute coronary thrombosis	Diabetes—quinidine death	16 hr.	Coronary thrombosis	++
54, M	Melanocarcinoma	Jejunal perforation, peritonitis, pulmonary embolization	36 hr.	Peritonitis	0
60, F	Rheumatic heart disease	Diabetes mellitus	3 days	Acute myocardial necrosis	++++
58, M	Rheumatic heart disease	Multiple pulmonary emboli with infarction	36 hr.	Pulmonary embolization	++++
63, F	Carcinoma of pancreas	Metastases	24 hr.	Undetermined	0
78, M	Acute coronary thrombosis	Arteriosclerosis	6 days	Coronary thrombosis	++
71, M	Acute coronary thrombosis	Bleeding duodenal ulcer (dicumarol)	10 days	Hemorrhage, coronary thrombosis	0
48, M	Cor pulmonale	Obliterative pulmonary arteriosclerosis	36 hr.	Acute heart failure	+
66, M	Cerebral accident	Arteriosclerosis, pulmonary edema	24 hr.	Cerebral accident	++
60, M	Carcinoma of bile duct	Operative transection of superior mesenteric vein	11 hr.	Hemorrhage	0
60, F	Rheumatic heart disease	Paroxysmal tachycardia	3 days	Coronary insufficiency	+++
71, M	Acute coronary thrombosis	Incarcerated bowel	24 hr.	Coronary thrombosis	++
47, F	Acute coronary thrombosis	Diabetes mellitus	6 days	Coronary thrombosis	+
61, F	Brain tumor	Postoperative bronchopneumonia	9 hr.	Operation	0
59, M	Carcinoma of lung	Postoperative hemorrhagic tracheobronchitis	13 hr.	Sepsis	0
40, F	Cirrhosis of liver	Rupture of esophageal varix	36 hr.	Hemorrhage	0
65, M	Cirrhosis of liver	Rupture of esophageal varix	5 days	Hemorrhage	0
35, M	Bleeding duodenal ulcer	Malignant hypertension, pulmonary infarcts	8 days	Hemorrhage	++++
74, F	Acute coronary thrombosis	Pulmonary infarcts	7 days	Coronary thrombosis	++++
52, F	Cardiac hypertrophy (unknown etiology)	Paroxysmal nodal tachycardia	48 hr.	Coronary insufficiency	++++
68, M	Hypertensive heart disease	Duodenal ulcer	24 hr.	Acute failure	++++

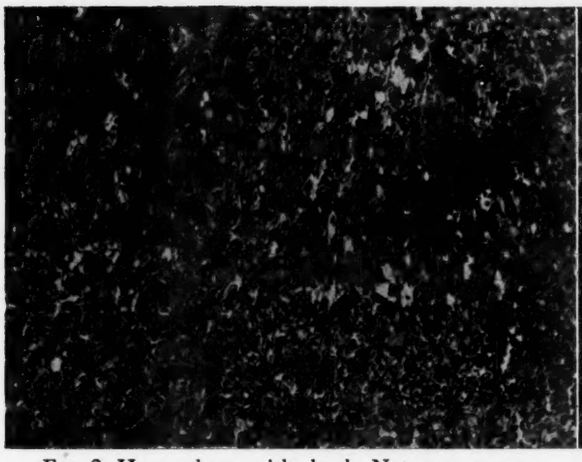

FIG. 2. Hemorrhage with shock. Note severe congestion, acute necrosis, architectural disruption and cellular infiltration. Normal liver cells are seen at the periphery.

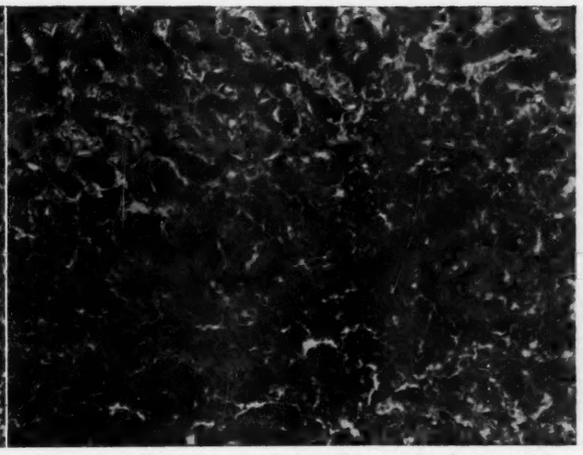

FIG. 3. Pulmonary embolization with shock. Note severe congestion, necrosis, cellular infiltration, architectural disruption and normal surrounding cells.

hospitalization he was discovered dead in bed. Autopsy revealed metastatic carcinosis. The liver grossly was the seat of diffuse multiple metastases; microscopically, there was definite pericentral necrosis. The findings differed from our shock cases in that there was relatively little architectural disorganization and a minimum of white cell infiltration. However, the similarity led us to include it in our series.

EFFECT OF CONGESTIVE HEART FAILURE

The role of congestive heart failure was of particular interest for three reasons: (1) Since our initial observations of central liver cell necrosis were in cases of acute coronary thrombosis, the possibility that these lesions might result from congestive failure was immediately suggested. (2) The older literature, especially the standard textbooks of pathology, uniformly attribute the presence of central liver cell necrosis to congestive heart failure. Occasionally, superimposed factors have been implicated but only in a secondary role. (3) In our cases there is a relatively high incidence of cardiac cases and congestive failure.

In order to evaluate the role of congestive heart failure two separate studies were made. In the first a group of twenty-five cases of prolonged and severe congestive heart failure were studied; secondly, in our group of 200 routine cases careful attention was paid to the presence or absence of clinical symptoms and signs of heart failure. It may be stated categorically that regardless of the degree of severity or duration of the congestive heart failure there was no acute

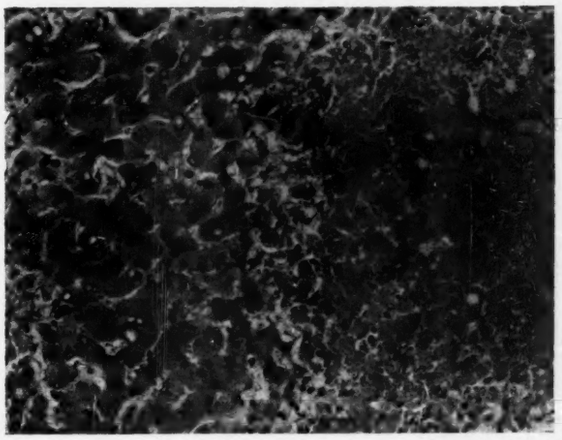

FIG. 4. Peritonitis with shock. Note sharp line of demarcation, acute necrosis, cellular infiltration and congestion.

central liver cell necrosis unless shock was superimposed for the necessary period of time. To cite some typical illustrations the following cases are briefly recorded:

CASE 93. This was a fifty-six year old male who had rheumatic chronic cardiovalvular disease with mitral insufficiency and stenosis. In addition he had hyperthyroidism which recurred following surgery and was subsequently treated with I^{131}. In the eight years preceding his death he had had sixteen admissions for repeated congestive heart failure and pulmonary edema. He died in severe congestion, in spite of which there was no evidence of central liver cell necrosis at autopsy.

CASE 123. This was a fifty-six year old male with chronic asthma and pulmonary fibrosis. He had been in right heart failure secondary to

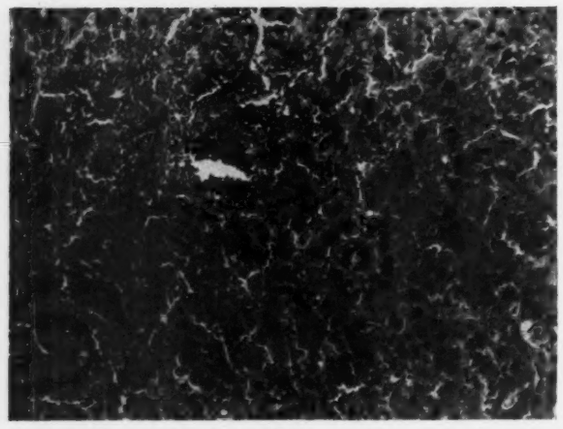

FIG. 5. Congestive heart failure without shock. Note severe congestion, edema and hemosiderin granules; but there is no necrosis.

this, with profound congestion, and had been given digitalis for fifteen years as well as weekly mercurial injections for four years preceding his death. He died in severe heart failure not attended by shock. The histologic examination revealed complete absence of central liver cell necrosis.

CASE 208. This was a fifty-seven year old male with cor pulmonale secondary to pulmonary fibrosis and emphysema. He had been in severe heart failure the last five years of his life, requiring digitalis and mercurials. In spite of the severity of the prolonged failure there was no central liver cell necrosis.

There are several possible reasons for confusion with congestive heart failure as the factor in the production of central liver cell necrosis. For example, the presence of acute central congestion immediately suggests the venous congestion associated with right heart failure, and the accompanying increased venous pressure. Moreover, as may be seen from our cases, a frequent mode of death in chronic congestive heart failure is from a shock-producing terminal complication such as pulmonary embolization, paroxysmal tachycardia or acute pulmonary edema. In addition, many causes of acute congestive failure, notably acute coronary thrombosis, are associated with a state of shock. In all such instances it seems apparent now that the presence of central liver cell necrosis results from the shock produced by the associated factors rather than from congestive heart failure *per se*.

From the physiologic aspect there is a marked difference between congestive heart failure and shock, since in peripheral shock there is no right heart failure, the venous pressure being reduced. From the pathologic aspect, although there may be a resemblance between these two conditions because of the central congestion present both in failure and in shock, there are several points of differentiation that are readily discernible on closer analysis. The chief difference is the absence of acute cellular necrosis. In congestive heart failure, especially of long-standing, the liver cords undergo a progressive atrophic change and may even disappear in the central areas but there is no evidence of acute necrosis. (Fig. 5.) Wallach and Popper[2] also reported histologic differences in the centrilobular changes in their cases of congestive heart failure as distinguished from those without congestive heart failure. The differences consisted chiefly of the absence of dissociation and fragmentation of cells in failure. Experimentally, the production of severe hepatic congestion did not result in hepatic damage even though large amounts of ascitic fluid were produced.[3]

PERTINENT LITERATURE

According to Davis[4] considerable disagreement exists concerning the anatomic lesions associated with shock, despite much work in this field. In the reported studies on the liver the alterations observed have not been consistent either quantitatively or qualitatively, although certain patterns and changes tend to recur. From our observations we believe that these changes are predictable if one takes into consideration the important time factor, i.e., the duration of the period of shock. As previously noted, many of the recorded observations have been made in animals in which it is frequently difficult to mimic the prolonged state of shock (twelve to ninety-six hours) which may occur in man. In animals the duration of shock frequently cannot be protracted beyond six to eight hours because of intervening death.

In a detailed study of the pathology of secondary shock Moon[5,6] concluded that there was a general pattern of the visceral changes in cases of shock irrespective of the nature of the precipitating factors. His cases included shock resulting from trauma, burns, poisoning, infections, anoxia, heat stroke, low atmospheric pressure and acute intra-abdominal conditions. The chief finding was dilatation and engorgement of capillaries and venules. This was associated with acute degenerative changes sometimes culminating in necrosis, most marked in

the liver, kidneys and myocardium. In the liver, degeneration and necrosis were regular features but their degree and distribution were inconstant. The degeneration varied from granular cytoplasm and vacuolation to necrosis. The criteria for the latter were disappearance of nuclei and the appearance of pyknosis and disintegrated cells. Moon also pointed out that anoxia is probably the most important factor in the production of these changes. In those cases in which the duration of shock appeared to be of prolonged duration the incidence of necrosis was markedly increased in frequency.

Darmady,[7] in a study primarily directed toward evaluation of renal anoxia in the traumatic uremia syndrome, carefully noted in the microscopic findings of his protocols the presence of central liver cell necrosis. All his cases were accompanied by severe shock and all were of relatively prolonged duration. Similarly Davidson et al.,[8] in a study of biochemical changes in peripheral vascular failure, also recorded in the microscopic findings of their protocols the presence of acute hepatic central necrosis in those patients who had had severe shock of prolonged duration.

Bywaters[9] studied the liver in traumatic cases. He indicated that the earliest degenerative changes, the appearance of eosinophilic globules, were observed after eight and one-half hours of shock. He further noted that the earliest necrotic changes that he could observe required twenty-four hours of shock. He concluded (1) that the liver lesions at death in all cases appeared to date from the time of the trauma and the initial appearance of shock, and (2) necrosis occurred in the most severely injured, as judged by the presence of shock. It is of especial importance in his report that, although he does not make a point of it, it is distinctly evident from his tables that the presence of central liver cell necrosis is definitely related to the presence and duration of shock. For example, of his ten patients one had a duration of shock of twenty-six hours and the other nine ranged from thirty-eight hours to eight days.

In a study of hepatic lesions in burns Wilson et al.[10] made a detailed correlation between the presence of hepatic changes and the duration of shock. Their findings in relationship to time are in the same range as ours. The earliest indications of liver degeneration were noted at twenty-one hours whereas actual necrosis of varying degree in the central zones, occasionally associated with hemorrhage, was seen in all cases lasting more than twenty-four hours. In those lasting more than 100 hours there was, in addition to necrosis, disorganization of the liver structure, collapse of the sinusoidal walls with hemorrhage and "nutmeg" appearance. Unfortunately, all their patients had been routinely treated previously with tannic acid, thus introducing a complicating factor since tannic acid itself has been implicated as a cause of central liver cell necrosis.

From the cardiac point of view Lambert and Allison[11] made a detailed study of the types of lesions encountered in chronic passive congestion of the liver. These lesions included capillary dilatation with atrophy of the cells, central degenerative changes, central fat accumulation, central necrosis and edema, and cardiac cirrhosis. It is of significance that they found central liver cell necrosis only in cases in which there was a severe and rapidly developing circulatory disturbance or, in modern terminology, shock. Of their three illustrative cases of central liver cell necrosis it is interesting to observe that two had acute coronary thrombosis and shock while the third was a rupture of an aortic aneurysm with profound shock.

More recently Clarke[12] observed a high incidence of central liver cell necrosis following myocardial infarction and noted the presence of shock in his cases. He emphasized the complete lack of correlation between central liver cell necrosis and congestive heart failure. The same author further noted that the frank, unequivocal changes of central liver cell necrosis became more readily apparent after twenty-four hours or more of infarction had elapsed.

From the experimental point of view we are again confronted with the time factor. Cameron and Mayes[13] ligated the hepatic artery of rabbits and found that even with such acute deprivation of oxygen definite cellular necrosis was first recognizable after fifteen hours.

PATHOGENESIS OF CENTRAL LIVER CELL NECROSIS IN SHOCK

Shock is accompanied by a fall in blood pressure, a diminution of blood flow, reduction in circulating blood volume, prolongation of the circulation time and a decrease in cardiac output. All these factors result in tissue anoxia; in fact, shock may be referred to as the anoxic phase of injury.

The liver is one of the most vulnerable tissues in the body in regard to sensitivity to oxygen deprivation. Further, because of the dual nature of its blood supply the liver is poorly fortified against abrupt changes in circulatory dynamics. Even under normal conditions the liver receives its oxygen under relatively low pressure because of the admixture of blood from the portal vein. Wiggers et al.[14] studied the portal blood pressure gradients in shock and concluded that the portal blood flow through the liver is reduced to an even greater extent than indicated by the changes in the systemic blood pressure. McMichael[15] showed that lowering of the blood pressure in shock proportionately lowers the oxygen content of the portal venous blood, making the liver more dependent on the hepatic artery. Bradley,[16,17] employing a technic of direct catheterization, demonstrated a specific role of the hepatic blood flow in cardiovascular dynamics under conditions of stress. In such situations the liver functions as a hemodynamic buffer since blood from its own vascular bed is shunted to other organs, thereby depleting its own source of oxygen.

In an attempt to compensate for the anoxia resulting from shock, various vasomotor mechanisms are evoked. This homeostatic response, which is a primary reaction to shock, consists of vasoconstriction of the smaller arterioles. Such vasoconstriction if sufficiently intense and prolonged may lead to tissue necrosis. Penner and Bernheim demonstrated acute necrosis in the upper[18] as well as in the lower[19] gastrointestinal tract as a result of severe, prolonged vasospasm.

One may then conjecture that the central liver cell necrosis we have observed results from shock, the accompanying anoxia and the compensatory vasospasm.

METABOLIC EVIDENCE OF LIVER INVOLVEMENT IN SHOCK

Many metabolic changes have been observed during shock and a large number of these have been demonstrated to result from liver dysfunction. In animals it has been shown that there is a marked reduction in the glycogen content of the liver, there is inability to store glycogen normally in the face of available glucose and the administration of insulin does not help the deficiency of glycogen storage.[20] Determination of oxygen consumption of liver slices also shows a marked decrease during shock.[21] Other changes include decreased deamination of amino acids, with a decrease in the synthesis of urea from alanine.[22] The peripheral manifestations of these metabolic changes include an increase in blood sugar, blood lactate, blood pyruvate and, perhaps even more significant, a rise in blood amino acid nitrogen.[23] The rise in amino acid content of the plasma distinctly implicates the liver. The rise in blood sugar, although undoubtedly due to epinephrine, is mediated through induced glycolysis in the liver. There is, in addition, evidence that the disorganization of liver metabolism in shock also involves destruction of enzyme systems. Involvement of the co-enzymes, cocarboxylase and cozymase, was shown by Wilhelm and Long.[24] More recently Shorr, Zweifach and Furchgott[25] have demonstrated a marked correlation of the VEM and VDM mechanisms in relation to the function of the liver in the development of shock.

SPECIFICITY OF THE LESION OF CENTRAL LIVER CELL NECROSIS IN SHOCK

Is the central liver cell necrosis that we have observed in shock a specific anatomic lesion? Or may this picture be produced by other factors? If so, is there a common pathogenesis?

Many liver poisons are known to produce central liver cell necrosis. The best known and most extensively studied are the chlorinated hydrocarbons, especially chloroform and carbon tetrachloride. A review of the literature on liver changes as a result of chloroform administration reveals in both dog and man[26] (1) that the microscopic findings are identical with those in shock and (2) the relationship of the picture to the time factor is also similar.

Cameron and Karunarante[27] described the changes in the liver of albino rats following administration of carbon tetrachloride. They found that not only are the anatomic changes identical with what we have described as a shock lesion but that the time necessary for the lesions to become manifest is of exactly the same duration as we recorded. Thus they found that after one hour there were no changes; after five hours there was some early sinusoidal congestion; at twenty-four hours they could see in the pericentral cells homogenization, eosinophilic staining, pyknotic or completely degenerated nuclei, marked congestion of the central areas frequently associated with definite hemorrhage, and cellular infiltration among the degenerating and necrotic cells. This lesion progressed so that after three days the cells in

the central zone had virtually completely disappeared.

With regard to the pathogenesis of the hepatic lesions resulting from carbon tetrachloride the observations of Wakim and Mann[28] are significant. They demonstrated that the earliest effect of carbon tetrachloride inhalation was immediate vasoconstriction of the intrahepatic circulation; only later was this followed by pathologic changes. Glynn and Himsworth,[29] using the black ink injection technic, demonstrated that the effects of carbon tetrachloride on the liver resulted from striking impairment of the blood flow which became progressively more marked. They found that marked restriction of the intralobular circulation occurred soon after the administration of carbon tetrachloride and persisted for many hours. Degeneration in the parenchyma, however, was not apparent until some hours after the appearance of the circulatory impairment. The lesions were confined to the centrilobular zone.

The centrilobular necrosis seen after poisoning with chlorinated hydrocarbons may now be attributed to ischemia of the centrilobular region rather than to direct action of the poison. In keeping with these findings previous experiments[13,15] have shown that when the large vessels of the liver are ligated ischemia is most marked in the central zone of the lobule.

It is quite obvious that central liver cell necrosis is not specific for shock. However, in view of the morphologic similarities and in view of the mechanisms involved in the production of the lesions by shock or certain liver poisons, it seems probable that central liver cell necrosis is a manifestation of anoxemia.

"BORDER-LINE" GROUP OF CASES

During this study we encountered twenty instances without sufficient changes to be considered central liver cell necrosis but with many pathologic features that were highly suggestive of necrosis. Some but not all of the well marked features of central liver cell necrosis were present. In general, the picture was as follows: Acute central congestion was present but as a rule did not extend as far into the periphery of the liver lobule. In the area of congestion there was a moderate amount of white cell infiltration. Nuclear and cellular changes, when present, were sharply limited to the pericentral area. Nuclear changes, which were more common, consisted chiefly of pyknosis, fading and

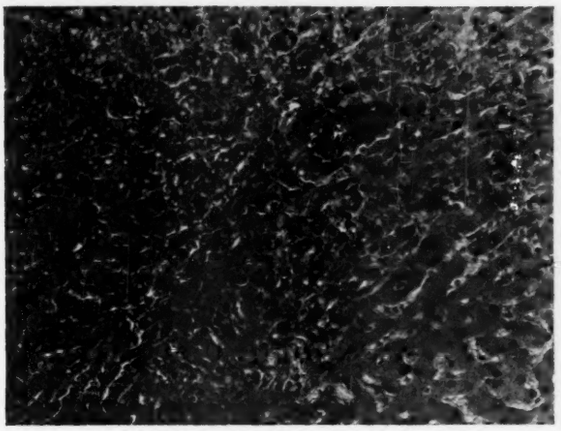

FIG. 6. Border-line case. Note acute congestion, staining changes, mild cellular infiltration and occasional necrotic cell.

occasional absence. The cells immediately surrounding the pericentral vein occasionally showed architectural disruption and, less frequently, mild necrosis.

A survey of the clinical aspects of these cases revealed very interesting data: (1) All were accompanied by shock and (2) the duration of shock in sixteen cases (80 per cent) was from ten to twenty-four hours; in only one instance did shock last more than twenty-four hours. In Table III these cases are included as part of the "negative" group. It is readily apparent from the table that the "border-line" cases had shock of less duration than the positive cases; and that the "border-line" cases comprise almost all of the cases with shock of only ten to twenty-four hours' duration. (Fig. 6.) Thus although the morphologic picture in this group is certainly not diagnostic, it in all probability indicates the nature and temporal relationships of the

TABLE III
RELATIONSHIP OF DURATION OF SHOCK IN POSITIVE, BORDERLINE AND COMPLETELY NEGATIVE CASES

Duration of Shock (hr.)	Positive Cases	Negative Cases	
		Borderline Cases	Completely Negative
0–10	2	3	38
10–24	5	16	2
24 or more	25	1	1
Totals	32	20	41
		61	

pathologic changes of necrosis. Preceding actual cellular necrosis there is congestion and sinusoidal dilatation, followed by cellular changes. Only after the factors accompanying shock have operated for a minimum time interval do the classical changes occur. This is further evidence of the importance of prolonged duration of shock in the production of central liver cell necrosis.

SUMMARY AND CONCLUSIONS

1. Central liver cell necrosis, as defined by (1) congestion, (2) eosinophilic staining, (3) nuclear changes, (4) cellular infiltration and (5) architectural disruption, all centrilobular in location, is definitely related to shock.

2. The duration of the shock is an important factor. Shock of less than twenty-four hours' duration infrequently results in central liver cell necrosis whereas shock of more than twenty-four hours' duration almost invariably does.

3. The cause of the shock is immaterial to the production of central liver cell necrosis.

4. Chronic congestive heart failure *per se* is not important in producing central liver cell necrosis.

5. Central liver cell necrosis is not a lesion specific for shock but is probably the end result of any prolonged vasospasm, anoxia and acute circulatory insufficiency.

6. The pathologic changes in central liver cell necrosis apparently begin with pericentral congestion and sinusoidal dilatation, and are then followed by staining alterations and cellular changes.

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Antibiotic Therapy of Friedländer Pneumonia*

WILLIAM M. M. KIRBY, M.D. *and* DANIEL H. COLEMAN, M.D.
Seattle, Washington

PRIOR to the introduction of streptomycin Friedländer pneumonia was a fatal disease in 70 to 80 per cent of cases.[1,2] With streptomycin therapy only three of fourteen patients so far reported in the literature have died, and cavity formation and chronic suppuration were infrequent among those who survived.[3]

Little information is available concerning the effectiveness of the newer antibiotics in the therapy of Friedländer pneumonia. The causative organism, Klebsiella pneumoniae, has been found to be highly susceptible to aureomycin, chloramphenicol and terramycin *in vitro* and in animal experiments. A dramatic response to aureomycin has been described in one patient who had shown no improvement after a week of penicillin therapy.[3] Three additional patients were stated by Dowling to have responded well to aureomycin.[4] No other cases of Klebsiella pneumonia treated with aureomycin have come to our attention and to our knowledge no cases in which patients were treated with chloramphenicol or terramycin have been reported.

From July, 1949, to July, 1950, eleven patients with Friedländer pneumonia were treated at the King County Hospital. These cases will be described, with emphasis on the response to therapy with sulfonamides, streptomycin, aureomycin and chloramphenicol.

RESULTS

Pertinent data concerning the eleven patients in the present series are summarized in Table I. For more detailed analysis the cases can be conveniently divided into two groups, namely, those who survived and those who did not. *In vitro* susceptibilities of nine of the strains of K. pneumoniae to aureomycin, chloramphenicol, terramycin and penicillin are presented in Table II.

Patients Who Died. Of the six patients who died four were known to be severe, chronic alcoholics. The other two were unable to give an adequate history but both were malnourished on admission. In four patients Friedländer's bacilli were isolated from the blood as well as from the sputum.

Four of the six patients (Table I, Cases I, II, IV and V) were in critical condition on admission and died within the first sixteen hours despite intensive therapy. Three received streptomycin and sulfonamides and the fourth was treated with aureomycin. The fifth patient (Table I, Case III) appeared to respond well to streptomycin and sulfadiazine but developed lower nephron nephrosis on the third day and was anuric thereafter. His death was probably related to this complication rather than to the pneumonia since his temperature was normal the last three days and there were signs of clearing of the pulmonary consolidation.

The sixth patient (Table I, Case VI) presented a complicated picture. Following five months of anorexia and weight loss he entered the hospital with a cough of one week's duration and signs of pneumonia at the right base. Blood and sputum cultures were positive for pneumococcus type 18. The patient was given chloramphenicol, 2 gm. daily, and his temperature fell to normal in two days. He remained disoriented, however, and became febrile again two days later. Aureomycin was administered intravenously in doses of 300 mg. every twelve hours but he failed rapidly and died four days later. A small amount of pus was removed from his right lower chest the day before his death and Friedländer's bacilli were isolated in pure culture. This was apparently a mixed infection; the initial response was due to suppression of the

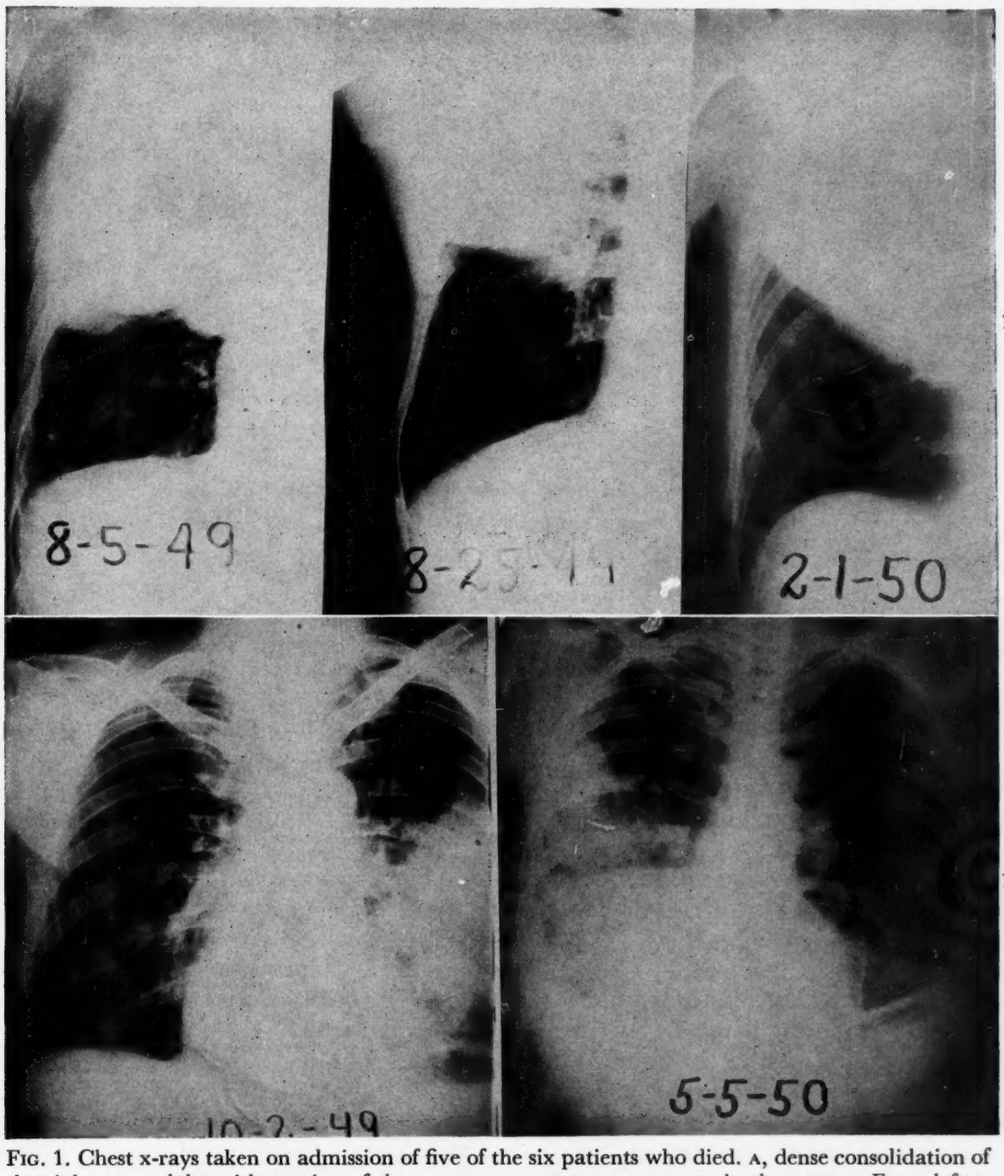
* From the Department of Medicine, University of Washington School of Medicine and the King County Hospital, Seattle, Wash.

TABLE I

SUMMARY OF PERTINENT DATA IN ELEVEN CASES OF FRIEDLÄNDER PNEUMONIA

Case	Date of Admission	Age (yr.)	Sex	Predisposing Factors	Day of Disease on Admission	Condition on Admission	White Blood Cells	Sputum Culture	Blood Culture	Course in Hospital Comments
Patients Who Died										
I M. B.	2/1/50	36	F	Alcoholism	7	Semi-comatose	1,900	F*	F	Treated with sulfadiazine and streptomycin; died in 16 hr.
II W. W.	8/25/49	65	M	Malnutrition	3	Seriously ill	5,000	F	F	Treated with sulfadiazine and streptomycin; died in 12 hr.
III F. J.	8/5/49	57	M	Alcoholism	5	Moderately ill	11,600 Polymorphonuclears 89%	F	F	Received sulfadiazine and streptomycin; temperature down after 4 days; lower nephron nephrosis developed on third day, with anuria thereafter; died on 7th day with blood urea nitrogen 152
IV F. S.	10/2/49	64	M	Alcoholism	3	Severely ill	1,900	F	Negative	Received penicillin, streptomycin, sulfadiazine, sulfamerazine; died in 9 hr.
V M. L.	5/5/50	59	F	Alcoholism	3	Severely ill	19,700 Polymorphonuclears 93%	F	F	Received aureomycin; died in 14 hr.
VI J.S.	6/3/50	74	M	Malnutrition	7	Severely ill	16,000	Pneumococcus	Pneumococcus type 18	See text; died after 7 days with Friedländer's empyema
Patients Who Survived										
VII S.W.	3/11/50	42	M	Alcoholism	1	Moderately ill	18,700 Polymorphonuclears 91%	F	F	Good response to chloramphenicol
VIII H. R.	9/6/49	54	M	Alcoholism	4	Moderately ill	15,000 Polymorphonuclears 87%	F	Negative	Received penicillin, streptomycin, and sulfadiazine; temperature normal after 1 wk.; gradual x-ray clearing in 2 mo.
IX E. F.	3/13/50	58	M	9-day course of penicillin therapy for syphilis was completed the day prior to onset	1	Moderately ill	8,500 Polymorphonuclears 78%	F	Negative	Good response to chloramphenicol, 1 gm. daily for 7 days; temperature normal on 4th day
X D. O'C.	2/12/50	59	M	Alcoholic	Unknown	Severely ill	7,800 Polymorphonuclears 80%	F Pneumococcus	Pneumococcus type 7	Started on chloramphenicol; penicillin and streptomycin given when meningitis developed; excellent response of both meningitis and pneumonia
XI J. K.	12/29/49	36	M	Severe alcoholic	7	Severely ill	11,000 Polymorphonuclears 89%	F	Negative	Course complicated by the presence of bronchopleural fistula and empyema; see text

*F = Friedländer's bacillus.



1A

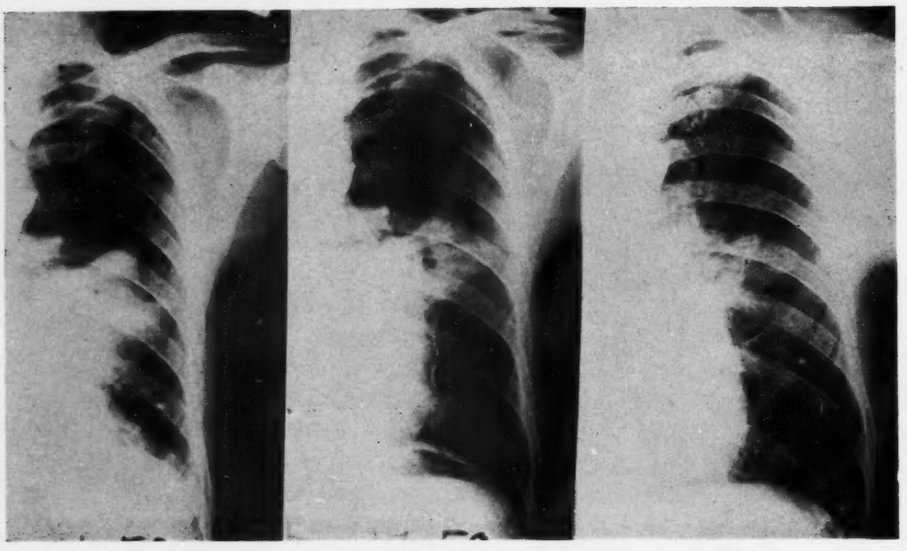
1B

FIG. 1. Chest x-rays taken on admission of five of the six patients who died. A, dense consolidation of the right upper lobe with sagging of the transverse septum was present in three cases. From left to right these were Cases III, II and I. (Table I.) B, chest x-ray of patient IV (Table I) is seen on the left and that of patient V is on the right.

pneumococci and the secondary rise in temperature was associated with the development of Friedländer's bacillus empyema. Because of the history of weight loss and anorexia this man may have also had some other underlying disease; unfortunately an autopsy was not performed.

Admission chest x-rays of five of the six patients who died are presented in Figure 1. Three patients (Cases I, II and III) had dense consolidation of the right upper lobe with sagging of the transverse septum, a picture which is highly characteristic of Friedländer pneumonia.[5] One patient (Case IV) had extensive involvement of the left lower lobe, and in Case V there was consolidation of the right middle and lower lobes.

In summary, four of the six patients who died had overwhelming infections at the time of entry and did not respond to intensive chemotherapy. In one the response to therapy was good but death was due to the development of lower nephron nephrosis. In the sixth case there was a mixed infection and death was associated with the development of Friedländer's bacillus empyema.



3/11/50 3/27/50 5/23/50

FIG. 2. Friedländer pneumonia, left upper lobe, in which there was an excellent response to chloramphenicol. Radiolucent areas in the third anterior interspace on the middle film are suggestive of cavitation. Despite early therapy residual infiltration and scarring were present two and a half months after the onset of the illness.

Patients Who Survived. Important points concerning the response of Friedländer pneumonia to chemotherapy are illustrated by the patients who survived; therefore each of these five cases will be summarized briefly.

Patient S. W. (Table I, Case VII), a forty-two year old white male, was a chronic alcoholic but denied drinking heavily during the week prior to admission. He entered the hospital with a six-hour history of chills, cough, rusty sputum and severe pleuritic pain in the left chest anteriorly. He had felt well the evening before and the symptoms appeared quite suddenly during the night. His temperature was 38.5°C. on admission and he was flushed and uncomfortable, breathing rapidly and guarding his left hemithorax. Dullness and a few crackling rales were noted over the left lower chest anteriorly and laterally. The white blood count was 18,700 with 91 per cent polymorphonuclears, and a direct smear of the sputum was loaded with encapsulated gram-negative rods. Friedländer's bacilli were cultured from both the sputum and blood. Chest x-ray showed a dense infiltration radiating out from the left hilum. (Fig. 2.) The patient was given 400,000 units of fortified procaine penicillin, 6 gm. of sulfadiazine and 1 gm. of streptomycin. However, he showed no improvement during the next sixteen hours and was therefore placed on chloramphenicol therapy, 6 gm. daily. There was marked subjective improvement within twenty-four hours, with a progressive decrease in cough and sputum. The pleuritic pain in the left chest persisted for six days and the temperature remained elevated to from 38° to 38.5°C. for a week. Sputum cultures remained positive for K. pneumoniae for five days and thereafter were negative. A blood culture taken on the fifth day of treatment was negative. Chloramphenicol was administered orally in doses of 6 gm. daily for seven days, following which 1 gm. was given daily for another four days. Cough and sputum decreased gradually and disappeared altogether approximately six weeks after the onset of the illness. An x-ray of the chest taken sixteen days after therapy was instituted showed some clearing of the dense infiltrations but there were radiolucent areas in the third left anterior interspace which were suggestive of cavitation. A film taken two months later showed further regression but considerable residual scarring and fibrosis persisted.

Patient H. R. (Case VIII) was a fifty-four year old chronic alcoholic who was admitted to the hospital from the city jail with a history of chills, fever, cough and rusty sputum of four days' duration. Rectal temperature was 39.5°C., and examination revealed signs of consolidation over the lower half of the right chest laterally and posteriorly. The white blood count was 15,000 with 87 per cent polymorphonuclears. Fried-

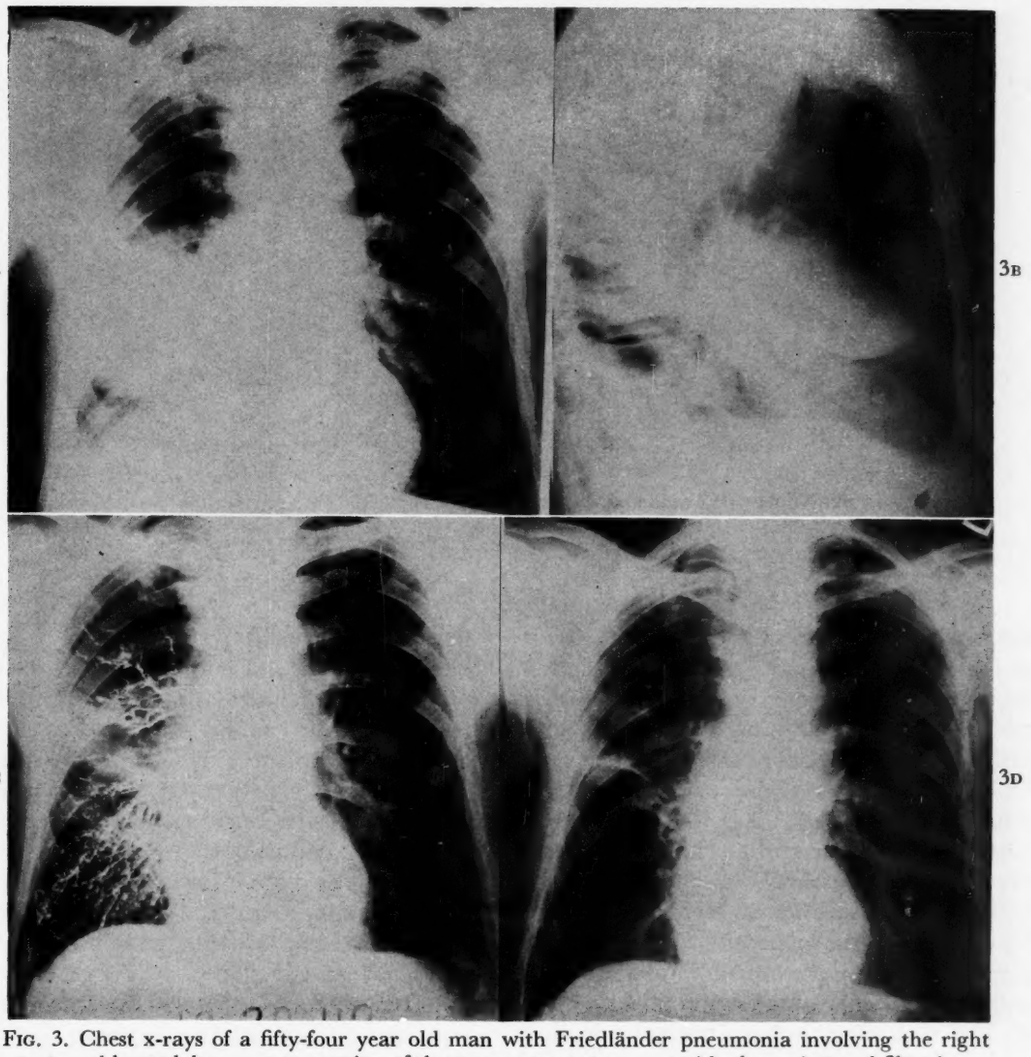
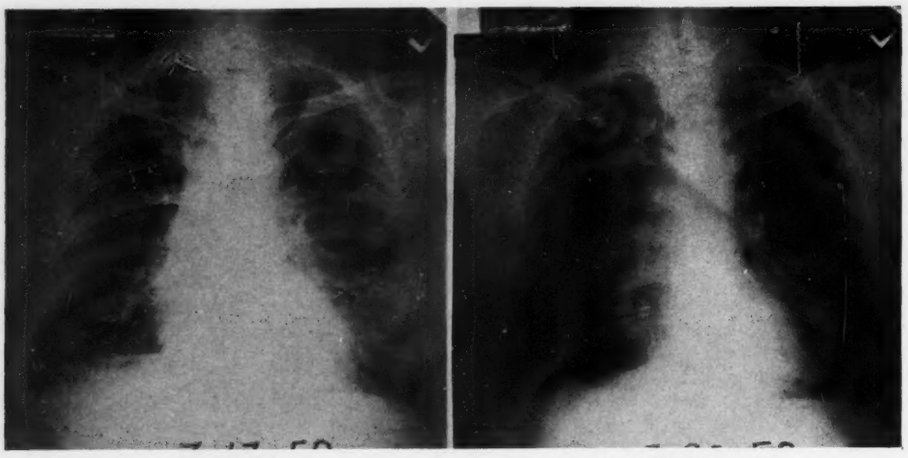


FIG. 3. Chest x-rays of a fifty-four year old man with Friedländer pneumonia involving the right upper and lower lobes. B, note sagging of the transverse septum. C, residual scarring and fibrosis are shown in a bronchogram made seven weeks later and in a film (D) taken four months after the onset of the illness. The patient was virtually asymptomatic at this time.

länder's bacilli were present in large numbers in the sputum. Chest x-ray (Fig. 3) revealed dense infiltrations of the right upper and lower lobes with marked sagging of the transverse septum. The patient was seriously ill and he was started at once on intensive chemotherapy consisting of 300,000 units of procaine penicillin, 2 gm. of streptomycin and 6 gm. of sulfadiazine daily. Within two days there was marked improvement although the temperature reached peaks between 38° and 39°C. daily for seven days. He was afebrile thereafter and the streptomycin, penicillin and sulfadiazine were discontinued after ten, thirteen and eighteen days, respectively. Cough and sputum gradually disappeared over a period of two months and the x-ray infiltrations cleared slowly although some residual scarring was still present four months after the onset.

Patient E. F. (Case IX) was a fifty-four year old white male who completed a nine-day course of penicillin therapy (9 million units) three days prior to admission. Cough, sputum and chilly sensations appeared the day penicillin was stopped and became progressively more marked during the next three days. On admission his temperature was 39.6°C., and he was coughing frequently, bringing up thick, viscid, yellowish white sputum. Harsh breath sounds and moist crackling rales were heard over both lower lung fields posteriorly. The white blood count was 8,500 with 78 per cent polymorphonuclears.



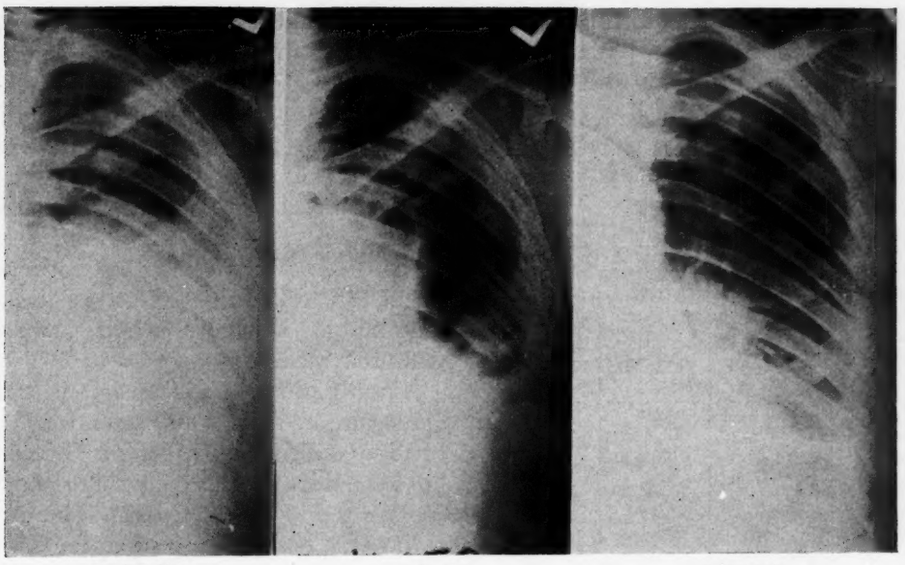
3/13/50 3/20/50

FIG. 4. Friedländer pneumonia of both lower lobes which developed after the patient had completed a nine-day course of penicillin therapy for syphilis. There was an excellent response to chloramphenicol, with almost complete clearing of the pneumonia within one week.

Friedländer's bacilli were present on direct smear and on culture of the sputum. K. pneumoniae were also recovered in pure culture from the peritoneal cavity of a mouse inoculated with the patient's sputum. X-ray of the chest revealed patchy infiltrations in both lower lung fields, more marked on the left. (Fig. 4.) The patient was immediately placed on chloramphenicol therapy, 1 gm. daily, which was continued for seven days. The temperature was elevated to 38.9°C. on the day after admission and to 38.7°C. on the third hospital day. He was afebrile thereafter, and signs and symptoms of pneumonia had cleared almost completely within a week after therapy was begun. This case was of unusual interest in that the pneumonia appeared after gram-positive organisms in the respiratory tract had presumably been suppressed by an intensive course of penicillin therapy. Another striking feature was the prompt response and complete clearing of the pneumonia, without residual fibrosis, despite the small doses of chloramphenicol which were administered.

Patient D. O'C. (Case x) was a fifty-nine year old chronic alcoholic who was admitted to the hospital acutely ill and unable to give a coherent history. His temperature was 40°C. He was breathing rapidly and dullness, rales and bronchial breathing were noted over the upper half of the right lung field anteriorly and posteriorly. There was no nuchal rigidity. The white blood count was 7,800 with 80 per cent polymorphonuclears. Blood culture was positive for pneumococcus type 7. Pneumococci and Friedländer's bacilli were present in large numbers on smear and culture of the sputum. He was begun on chloramphenicol, 2 gm. daily by mouth, but was found the next day to have a stiff neck. The spinal fluid contained 432 cells per cu. mm., of which 90 per cent were polymorphonuclears. Pneumococci were present on smear and culture of the spinal fluid. Chloramphenicol was discontinued and the patient was given 1 million units of penicillin every two hours and 3 gm. of streptomycin daily, both by the intramuscular route. There was steady improvement with the temperature gradually subsiding to normal in one week and signs of meningitis and pneumonia clearing rapidly during the same period. Streptomycin was continued for seven days and penicillin was administered in gradually decreasing amounts for three weeks. Convalescence was essentially uneventful, and except for occasional headaches and dizzy spells the patient has remained well. It seems probable that the pneumonia in this case represented a mixed infection. Penicillin appeared to play a major role in suppressing growth of the pneumococci while streptomycin was of value in controlling invasion by the Friedländer bacillus.

Patient J. K. (Case xi) was a thirty-six year old white male cook, a severe alcoholic, who had been ill for seven days with cough, sweats, aching pain in the left chest, malaise and anorexia. His sputum had been blood-streaked for two days. On admission the temperature was 39.2°C., and dullness to flatness, diminished breath sounds



12/29/49 1/4/50 3/13/50

FIG. 5. A case of Friedländer pneumonia complicated by a bronchopleural fistula and empyema. With open drainage and antibiotic therapy, cultures of the empyema fluid and sputum turned negative, and the fistula and empyema cavities gradually became obliterated.

and bubbling rales were observed over the lower half of the left chest both anteriorly and posteriorly. The white blood count was 11,000 with 89 per cent polymorphonuclears. Chest x-ray revealed a homogeneous density over the lower half of the left lung field. (Fig. 5.) Many gram-negative bacilli with large capsules were seen on direct smear of the sputum and an almost pure culture of K. pneumoniae was obtained on culture. Aureomycin therapy, 3 gm. daily by mouth, was begun immediately and thoracenteses were performed daily for five days, with removal on each occasion of 300 to 800 cc. of moderately thick pus from which Friedländer's bacilli were isolated in pure culture. Chest x-ray on January 4, 1950, revealed a large amount of air in the left pleural space, indicating the presence of a bronchopleural fistula since no air was introduced during the aspirations. Despite aureomycin therapy and repeated aspirations the patient's temperature remained elevated to between 38° and 39°C., and cultures of the sputum and empyema fluid remained positive for Friedländer's bacilli. On the fourteenth hospital day a rib resection and open drainage of the empyema were performed. Aureomycin was discontinued the next day and thereafter the patient received sulfadiazine, 6 gm. daily, and streptomycin, 1 gm. daily, for three weeks. Cultures of the sputum and empyema fluid became negative five days after open drainage was established. The temperature fell to normal over a period of one week. The bronchopleural fistula remained open for over two months but eventually it closed as the lung re-expanded (Fig. 5), and after four months the external wound stopped draining and healed. The patient has continued to cough intermittently. On one occasion there was gross hemoptysis. He has refused to cooperate with further studies.

In summary, this severe alcoholic with Friedländer pneumonia, empyema and a bronchopleural fistula showed little response to aureomycin therapy and repeated aspirations. Following open drainage and therapy with streptomycin and sulfadiazine, the lung gradually re-expanded and the fistula and empyema were obliterated. Symptoms indicative of chronic pulmonary damage have persisted.

COMMENTS

The classical features of Friedländer pneumonia are well illustrated by the present series of patients. The disease occurs predominantly in males, the usual ratio being 4:1; in the present series nine were males and two were females. The predilection of the Friedländer bacillus for alcoholics is well known, and eight of these patients had a history of chronic alcoholism. Dense consolidation of pulmonary tissue with sagging and bulging of interlobar septa, which is virtually diagnostic from an x-ray standpoint, was present in approximately half of our patients. (Figs. 1 to 5.) Rapid destruction of pulmonary

tissue with residual fibrosis and suppuration occurred in the majority of the patients who survived although this feature was undoubtedly modified by antibiotic therapy.

Most of the patients were in poor physical condition when they contracted the pneumonia.

TABLE II
RESULTS OF SENSITIVITY TESTS PERFORMED ON NINE STRAINS OF K. PNEUMONIAE *

Case	Aureomycin	Chloramphenicol	Terramycin	Penicillin
I M. B.	1.5	3	3	>100
II W. W.	12	50	12	>100
III F. J.	....	..	...	...
IV F. S.	3	12	6	50
V M. L.	100	50	200	25
VI J. S.	....	..	...	...
VII S. W.	1.5	3	3	12
VIII H. R.	3	12	6	50
IX E. F.	6	3	6	100
X D. O'C.	12	6	12	>100
XI J. K.	1.5	3	3	50

* Concentrations of aureomycin, chloramphenicol and terramycin are expressed in micrograms per cc. and penicillin in units per cc.

In addition some did not come under medical supervision until the disease had become overwhelming in severity. These features are difficult to control and make it likely that the mortality rate from Friedländer pneumonia will remain high despite the advances which have been brought about by chemotherapy.

One of the main objectives in treating these patients was to compare the efficacy of streptomycin with that of newer antibiotics such as aureomycin and chloramphenicol. The difficulty in obtaining information which is statistically valid concerning this problem is well illustrated by the fact that although streptomycin has been available for six years results obtained with this antibiotic in the treatment of Friedländer pneumonia have been reported in only fourteen cases.[3]

In vitro studies of nine strains of K. pneumoniae isolated from our patients indicated that with one exception (Table II, Case V) all were quite susceptible to aureomycin, chloramphenicol and terramycin. The susceptibility of a given strain was in general roughly the same for all three of the antibiotics, i.e., variations of more than one tube were unusual. In addition four of the strains were inhibited by concentrations of penicillin (50 units per cc. or less) which are attainable clinically when large doses are administered.

Two patients made an excellent response to chloramphenicol. One, who was very ill and had a positive blood culture, received 6 gm. daily and is now clinically well although residual scarring and fibrosis persist. The other patient, who was less severely ill, received only 1 gm. daily and recovered completely within one week. The response in these patients appeared to be at least as good as that which might be expected from streptomycin and to our knowledge these are the first cases treated with this antibiotic which have been reported.

Three patients received aureomycin. In two patients (Cases V and VI) death occurred early in the illness and the result could not be evaluated. In the third patient (Case XI) the pneumonia was complicated by a bronchopleural fistula and empyema. There was some subjective improvement on aureomycin therapy but the empyema fluid and sputum remained positive for K. pneumoniae until open drainage was established. Thereafter the patient received streptomycin and sulfadiazine and the cultures became negative within a few days.

Taken together, the sensitivity studies and the clinical response observed in the patients indicate that aureomycin and chloramphenicol are probably comparable to streptomycin in the therapy of Friedländer pneumonia. It would at least appear to be safe to treat patients with these antibiotics, and it can be hoped that a sufficiently large number of cases will eventually be accumulated so that a comparison of various regimens will be possible.

SUMMARY AND CONCLUSIONS

1. During a one-year period from July, 1949, to July, 1950, eleven patients with Friedländer pneumonia were treated at the King County Hospital. Nine were males. Eight were chronic alcoholics. In one patient symptoms appeared

immediately following a nine-day course of penicillin therapy for syphilis.

2. Six of the eleven patients died. Four had overwhelming infections and died within sixteen hours despite intensive chemotherapy. In one patient death was due to lower nephron nephrosis. The sixth patient had a mixed infection and death was probably due to Friedländer's bacillus empyema. Four of the patients who died had positive blood cultures while only one of the patients who survived was bacteremic.

3. Of the five patients who survived two responded well to chloramphenicol, and the others received various combinations of streptomycin, sulfadiazine, aureomycin, penicillin and chloramphenicol. In one patient, whose pneumonia was complicated by a bronchopleural fistula and empyema, there was little improvement on aureomycin therapy plus repeated aspirations. Following open drainage the infection responded promptly to streptomycin and sulfadiazine. In three patients there was residual scarring and fibrosis following recovery from the acute illness.

4. Because of the generally poor physical condition of individuals who contract Friedländer pneumonia and the frequent delay in starting therapy it is probable that the mortality rate from this disease will remain high. However, in the patients who were in relatively good physical condition and in whom treatment was started early the response to chemotherapy was remarkably good.

5. On the basis of *in vitro* sensitivity tests and the clinical response observed in the present series of patients it would appear that aureomycin and chloramphenicol are probably as effective as streptomycin in the therapy of Friedländer pneumonia.

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Clinical and Pharmacologic Studies with Allylmercaptomethyl Penicillin (Penicillin "O")*

CHARLES V. ADAIR, M.D., WILLIAM G. WOODIN, M.D. *and* PAUL A. BUNN, M.D.
with the technical assistance of Leonard Canarili, B.S.

Syracuse, New York

RECENT communications have described the chemistry of allylmercaptomethyl penicillin and have indicated that it can be safely substituted for benzyl penicillin (penicillin G) in instances of hypersensitivity reactions to the latter.[1–4] This report will describe further experiences and observations with allylmercaptomethyl penicillin (penicillin "O"). Its pharmacologic properties in the human have been studied. It has been used to treat a series of miscellaneous infections, some of extraordinary severity, caused by penicillin susceptible organisms. Finally, individuals presently or in the past suffering from an allergy to benzyl penicillin (G) administered in a variety of vehicles have been given penicillin "O." Some of the latter were not necessarily ill with an infection at the time of its administration.

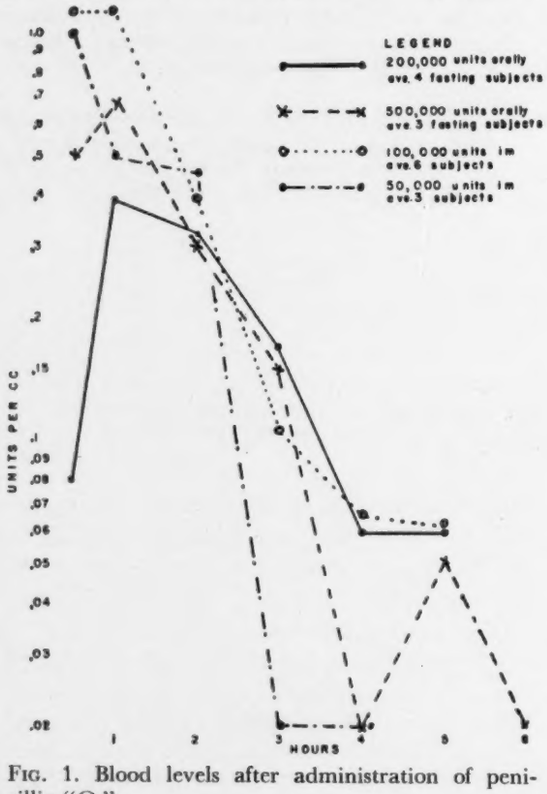

FIG. 1. Blood levels after administration of penicillin "O."

PHARMACOLOGY

Absorption, distribution and excretion of many penicillins are reasonably well understood and there is unanimity of opinion concerning what is expected to occur in the human after any single dose or repeated doses of penicillin G in particular.[5–7] Similar information is appearing concerning penicillin "O"[3,4] and this study presents further description of its pharmacologic properties in the human.

Serial blood concentrations in fourteen normal subjects have been measured following oral and/or intramuscular administration of penicillin "O." (Fig. 1.) By both routes peak concentrations were observed within sixty minutes after administration. Following 200,000 or 500,000 unit doses in water by mouth to fasting healthy subjects peak concentrations in serum ranged from 0.25 to 1.35 units per cc. and levels above 0.03 units were present for five hours in the majority of the subjects studied. Higher peak levels were attained following intramuscular injections of 50,000 and 100,000 units (up to 1.4 and 2.85 units per cc., respectively) but duration of measurable levels was not more prolonged than those after large oral doses. Levels

* From the Department of Medicine, State University of New York, Medical Center at Syracuse. Patients in this study were hospitalized at the University, Memorial and City Hospitals, Syracuse, New York. This investigation was supported in the main by a grant from Bristol Laboratories, Inc.

attained after either route of administration were not essentially different from those observed with penicillin G. In three cases urinary excretion of penicillin "O" has been determined. Concentration in the urine was found to be similar to that recovered after single doses of penicillin G and excretion proceeds at an identical rate. Between 33 and 50 per cent of the total single intramuscular dose and approximately 10 per cent of an oral dose were recovered within eight hours. In only one instance was spinal fluid concentration determined after a single large intramuscular dose; it was identical with that expected after a similar parenteral injection of penicillin G.

It was evident from these few studies that the speed and degree of absorption of penicillin "O" from two areas of administration (muscle and gastrointestinal tract), its distribution in serum and spinal fluid with each route and its excretion rate and amount were essentially identical with that expected after the administration of penicillin G.

No gross evidence of toxicity in normal subjects receiving single doses of penicillin "O" was experienced although detailed functional studies of kidney, liver and other body systems were not undertaken. This observation became somewhat more secure with the knowledge that patients tolerated as much as 12,000,000 units daily without untoward event. Soreness and inflammation at the site of intramuscular inoculation was not a serious complaint nor has there been objective evidence to indicate that penicillin "O" was a greater irritant than any form of G in aqueous solutions. Instillation into body cavities was tried in only one patient. In this instance 400,000 units were injected intrapleurally and provoked no reaction. By mouth and dissolved in tap water penicillin "O" was bitter but was not intolerable, and no instance of nausea, vomiting or diarrhea occurred following this route of administration.

CLINICAL EFFICACY

A series of fourteen patients with miscellaneous infections were treated entirely or in major part with this substance.* In addition, two other patients without manifest infections who were suffering from other illnesses were given "O" prophylactically. Clinical features of the sixteen patients are recorded in Table I.

* Penicillin "O" (Cero-cillin) was generously supplied by Upjohn and Co.

With but one possible exception, clinical and bacteriologic response was in every way as satisfactory as would be expected with penicillin G. The single exception, a case of pneumococcic meningitis, was acutely and morbidly ill on admission to the hospital and died in eight hours without recovering from shock after receiving only four doses of penicillin "O." Two illustrative patients are described:

CASE 1. R. A., a forty-two year old white male, had subacute bacterial endocarditis due to alpha hemolytic streptococci isolated by blood culture. The organism was sensitive to 0.03 u./cc. of penicillin. He was treated with 50,000 units of penicillin "O" every three hours intramuscularly. He was afebrile in forty-eight hours. After the initial satisfactory response with penicillin "O" he was switched to penicillin G and completed a total course of fifty-three days of penicillin. He was discharged as cured but died two months later in cardiac failure. At autopsy there were numerous healed and sterile vegetations on the aortic valve with perforation of one cusp. Cause of death was not infectious and it was presumed that he died because of mechanical cardiac difficulties.

CASE 10. E. R., a forty-three year old white housewife had pneumococcic type 10A meningitis with bacteremia but no pneumonia. She was in coma for eight hours prior to onset of therapy. Because of a history of giant urticaria following penicillin injections for ethmoid sinusitis six months previously, penicillin "O" was used initially in a dosage schedule of 1,000,000 units every two hours intramuscularly; no intrathecal therapy was administered. She remained comatose, restless and febrile for ninety-six hours. Thereafter she made an uneventful recovery with no complications or sequelae. Blood and cerebral spinal fluid levels of penicillin "O" two hours after an injection of 1,000,000 were 6.4 u./cc. and 0.3 u./cc., respectively.

After fourteen days of therapy with "O" a single large dose of G was administered; a definite urticarial wheal appeared promptly at the site of injection. Two and three days later single doses of 50,000 to 1,000,000 units of G were given intramuscularly without event.

PATIENTS WITH HYPERSENSITIVENESS TO PENICILLIN G GIVEN "O" AS A SUBSTITUTE

Twenty-five patients, with or without infections, showing hypersensitivity reactions to

penicillin G were subsequently treated with penicillin "O." In twenty-one of these the reactions to G were actually observed. (Table II.) The four additional patients gave only a previous history of rash or urticaria to G and are included in Table I. Brief abstracts of three cases are described:

CASE 1. R. N., a forty-seven year old white male, was morbidly ill with a perforated appendix and acute peritonitis. After three days of therapy with penicillin and streptomycin a widespread and serious urticarial rash was noted. Penicillin "O" was substituted and streptomycin continued for twenty-six hours. The rash cleared. A different commercial brand of penicillin G was substituted thirty hours after the first dose of "O" and no further rash was noted.

CASE 3. H. D., a fifteen year old white male had acute osteomyelitis. Fourteen days after the start of therapy with penicillin G he developed a pruritic maculopapular rash over the trunk and extremities. A progressive rise in eosinophilia from 6 to 22 per cent was noted during this time. Penicillin "O" was substituted for three days; eruption cleared in thirty hours. A single dose of penicillin G (50,000 units) was then given with recurrence of the same rash four hours after the dose. Penicillin "O" was again administered, the rash subsided and there was no further reaction. Penicillin G (same commercial brand) was substituted once more after seven days of therapy with penicillin "O" and no rash occurred. His eosinophilia subsequently fell to 7.5 per cent.

CASE 4. W. H., a forty-one year old white

TABLE I

THERAPEUTIC RESULTS WITH PENICILLIN "O"

Case No.	Name	Age	Sex	Hypersensitivity to Penicillin G	Diagnosis	Dose (units)	Duration	Comment
1	F. A.	42	M	0	Subacute bacterial endocarditis—alpha streptococcus	50,000 q 2 hr. IM	10 days	Afebrile in 48 hr.; completed remaining treatment (53 days) with penicillin G
2	L. C.	21	F	0	Cellulitis of jaw, Staph. aureus	100,000 q 8 hr. IM	5 days	Prompt and complete healing with incision and drainage on second day
3	J. C.	62	M	0	Pneumococcic meningitis	1,000,000 q 2 hr. IM	8 hours	Comatose, died; no effect
4	A. F.	27	M	0	Pneumococcic pneumonia type 1	200,000 q 8 hr. oral	5 days	Afebrile in 36 hr.
5	M. C.	70	F	0	Pneumococcic pneumonia type 3	100,000 q 8 hr. IM	10 days	Bacteremia; afebrile in 48 hr.; complete clearing by x-ray in three weeks
6	A. K.	68	M	0	Lobar pneumonia, no pneumococci isolated	400,000 q 8 hr. oral	4 days	Afebrile in 36 hr.; clearing by x-ray in three weeks
7	W. G.	80	M	0	Pneumococcic bronchopneumonia	400,000 q 8 hr. oral	5 days	Afebrile in 24 hr.; no relapse
8	J. K.	41	M	0	Acute periodontitis and maxillary sinusitis	500,000 q 12 hr. oral	5 days	Afebrile in 24 hr.; rapid healing of infection
9	S. S.	51	M	Rash 1 yr. previously, presumably from penicillin G (aqueous)	Chronic draining sinus and presternal abscess due to Staph. aureus (resistant to 1.0 u penicillin/cc.)	1,000,000 q. 2 hr. IM	8 days	Sinus excised and abscess drained with healing; penicillin G used later without reaction
10	E. R.	43	F	Previous giant urticaria	Pneumococcic meningitis type 10A	1,000,000 q 2 hr. IM	17 days	Afebrile and out of coma in 96 hr.; adequate blood and CSF level; no reaction to penicillin G given later
11	C. B.	61	F	0	Stasis dermatitis, varicose ulcers	100,000 TID oral	7 days	Healing of ulcer in conjunction with rest and elevation
12	C. S.	78	F	0	Infected carcinoma of nose	400,000 TID oral	15 days	Decrease in inflammatory reaction
13	M. F.	78	F	0	Arteriosclerotic ulcers of toes with infection	200,000 TID oral	7 days	Healing progressed slowly
14	O. A.	33	F	Generalized urticaria 3 yr. before	Septic sore throat due to B. hemol. strep.	200,000 IM 4 times daily	6 days	Negative skin and patch tests to both penicillin G and O; no reaction noted upon readministration of penicillin G; throat cleared promptly
15	A. Y.	33	F	Generalized urticaria from aqueous G 6 mo. previously	Trauma; compound comminuted fracture	Dose unknown	.....	Used prophylactically; negative skin and patch tests to penicillin G and O; urticaria had persisted 6 mo.
16	M. O.	88	F	0	Emphysema, A.S.H.D. with heart failure	50,000 q 4 hr. IM	11 days	Used prophylactically

TABLE II

RESULTS WITH USE OF PENICILLIN "O" IN PATIENTS HYPERSENSITIVE TO PENICILLIN G

Case No.	Age	Sex	Manifestation Hypersensitivity to Penicillin G	Diagnosis	Dose of "O" (units)	Duration of Therapy	Skin or Patch Tests		Subsequent Reaction to G and Comment
							"O"	G	
1	47	M	Severe giant urticaria after 7 days' therapy with aqueous G	Ruptured appendix with peritonitis	75,000 q 2 hr. IM	26 hours	....	....	No reaction after 30 hr. of therapy with O; therapy with G continued for 10 days thereafter
2	51	M	History of rash after IM therapy with aqueous G	Chronic osteomyelitis (Staph. aureus)	50,000 q 3 hr. IM.	6 days	....	....	No reaction with local re-administration of G after 6 days
3	15	M	Pruritic generalized maculopapular rash with IM therapy with G	Acute osteomyelitis	100,000 q 3 hr. IM.	Total 6 days (2 courses)	Neg.	Neg.	Recurrent rash after 3 days; none after 7, and G continued thereafter for one month
4 &	41	M	Pruritic rash while receiving aqueous G	Pneumococcic pneumonia	50,000 q 3 hr. IM.	3 days	Pos.	Pos.	Rash did not disappear until O was discontinued; failure of substitution therapy
5	20	M	Generalized urticaria after procaine G in oil for 3 days	Acute otitis media	50,000 IM	1 dose	Pos.	Pos.	Marked immediate and delayed reactions after skin tests with G and O; initial urticaria persisted 6 weeks; failure of substitution therapy
6	53	M	Urticaria, site of injection; procaine G in oil after 3rd daily dose	Bronchitis, ethmoiditis	50,000 q 3 hr. IM	3 days	Pos.	Pos.	Milder local reaction after 6 days
7	49	M	Urticaria, site of injection with procaine G in oil after 3rd daily dose	Asthma	Dose unknown	.....	Pos.	Pos.	No reaction to re-administration of G; had had similar local reactions to aqueous penicillin in 1947 and 1949
8	50	M	Giant wheals site of injection and generalized maculopapular rash after 7 days therapy with aqueous G	Luetic aneurysm popliteal artery with rupture	100,000 q 3 hr. IM	7 days	....	....	No reaction after one week; resubstitution of G continued for 3 weeks
9	46	M	Maculopapular rash on 7th day of therapy with aqueous G	Acute tabes dorsalis	100,000 q 3 hr. IM	7 days	....	....	No reaction after one week; re-substitution of G continued for 3 weeks
10	50	M	Generalized maculopapular rash under therapy with aqueous G	Multiple infected lacerations	100,000 q 3 hr. IM	21 days	....	....	No reaction; therapy with G continued 7 days
11	21	F	Severe urticaria site injection, procaine G in oil	3rd degree burns with large abscesses both thighs	200,000 q 3 hr. IM	10 days	....	....	No reaction—single dose of G; local reaction disappeared during administration of O
12	55	F	Generalized urticaria from both G and procaine G	Pneumococcic sinusitis	200,000 b.i.d. IM	3 days	....	....	Urticaria cleared during therapy with O; G not given again
13	50	M	Generalized rash and fever 5 days therapy with aqueous G	Infected wound over compound, comminuted fracture	100,000 q 3 hr. IM	7 days	....	....	No reaction; G continued for 7 days more
14	40	F	Urticaria site injection after 42 doses of 200,000 G in water	Asthma, infective type, with exacerbations	200,000 q 4 hr. IM	5 days	Neg.	Neg.	Acute edema nasal mucous membrane within 1 hour after 3 separate courses of G, 6 weeks apart
15	48	M	Urticaria after 1 dose procaine G in oil 9 months previously	Infective type asthma	50,000 q 3 hr. IM	? days	Neg.	Neg.	No reaction with re-administration of 1 dose of G; penicillin 2 yr. ago resulted in similar local urticaria; present reaction had persisted 9 months
16	43	M	Maculopapular rash	Acute sinusitis	50,000 q 3 hr. IM	.....	Neg.	Neg.	No reaction
17	59	M	Mild generalized rash and fever	Bronchogenic carcinoma with postoperative pleural infection	400,000 intrapleurally	1 dose	....	....	No cutaneous reaction with O intrapleurally; G not re-administered
18	31	M	Urticaria after 7th dose procaine G in oil	Sinusitis, acute etiology unknown	None	.....	Pos.	Pos.	G not re-administered; weekly positive passive transfer reactions to both O and G; immediate reaction only to O; immediate and delayed reaction to G; lesion at site of skin test with G extended to involve forearm and hand over a period of 24 hours; site of O skin test negative at 24 hours
19	40	F	Immediate rash after topical administration of G to surgical wound	Meningocele middle ear	100,000 q 2 hr. IM	4 days	....	....	G not re-administered; O used prophylactically
20	53	M	Spreading pruritic rash on 11th day of therapy with aqueous G	Myocardial infarction	200,000 b.i.d. IM	15 days	Neg.	Neg.	G not re-administered; O used prophylactically
21	55	F	Large wheal at site of injection procaine penicillin	Multiple fractures	100,000 q 3 hr. IM	16 days	....	....	G not re-administered; O used prophylactically

male, had severe pneumococcic (pool 3) pneumonia with bacteremia that had failed to respond to terramycin. Penicillin G produced a prompt crisis with concomitant improvement in his clinical condition, but after ten days a generalized pruritic maculopapular rash was observed. Penicillin "O" was substituted but the rash did not disappear until penicillin "O" was stopped. Skin testing with both penicillin "O" and G gave positive reactions to both.

In the majority of patients known to have had or who developed a delayed type of reaction to penicillin G during treatment, a change to penicillin "O" was accomplished with diminution or, generally, total disappearance of the allergic phenomenon. In this series it was possible to substitute "O" for G safely in twenty-two of twenty-five patients hypersensitive to G. It was also possible to re-substitute G after a short interval of from one and one-half to fourteen days in an appreciable number of individuals receiving "O" and no recurrence of the original reaction to G was observed. This was practicable in fourteen of fifteen patients in this series in whom the re-substitution was attempted.

BRIEF SUMMARY OF HYPERSENSITIVITY PHENOMENA TO PENICILLIN AND A DESCRIPTION OF SOME EXPERIENCES WITH SUBJECTS ALLERGIC TO PENICILLIN G

Untoward Reactions to Penicillin. Untoward reactions to penicillin are few. Essentially they fall into three groups: (1) the local reaction after instillation of penicillin into joint spaces and muscles as well as into pleural, pericardial and spinal cavities, and the contact type of cutaneous reaction following habitual handling of penicillin preparations, (2) the "id" phenomenon and (3) the delayed or acute anaphylactoid reaction. The delayed reaction is by far the most common and is of most concern in therapy of acute infections. The reaction is generally considered to be due to hypersensitivity to a salt of benzylpenicillin or to its *in vivo* protein-bound material. Immediate and acute reactions with urticaria are occasionally observed and may be accompanied by angioneurotic edema, vomiting, abdominal cramps, dyspnea, syncope and, on occasion, death.[8] Concomitants of the delayed type of reaction may be a rash, arthralgia, malaise, fever and generalized lymphadenopathy.

The "id" phenomenon is uncommon. A resemblance has been noted between cutaneous reactions following penicillin therapy and lesions associated with certain fungus diseases. Cormia, Lewis and Hopper show that there is a common antigen between penicillin and certain of these pathogenic fungi and suggest that the reactions to penicillin in man may be due to prior sensitization to the proteins of such pathogenic fungi.[9,10]

Reports in the literature lead one to expect allergic reactions in from 0.5 to 5.7 per cent of all patients treated with penicillin.[11–15] Lepper, Dowling and co-workers report allergic reactions in 1.2 per cent of 598 patients treated with aqueous crystalline penicillin, 2.7 per cent of 557 patients treated with crystalline penicillin in oil and beeswax and 1.4 per cent of 148 patients receiving procaine penicillin in oil. Their incidence was highest in patients receiving very large doses of penicillin and in those receiving penicillin over a long period of time.[15] No breakdown of incidence of the three types of reactions was reported.

Contact Dermatitis and "id" Phenomena. A small series of patients demonstrating either the "id" phenomenon or the contact type of allergic manifestation have been studied. (Table III.)

In four individuals demonstrating contact dermatitis, i.e., following habitual handling of the material, or from local application of various penicillin preparations, ointments and the like, or after instillation locally into body cavities, "O" was not a substitute for G in any case in the sense that a diminution or absence of further reactions was observed. The following patient is illustrative of all:

CASE 1. S. S., a twenty-six year old nurse, developed vesicular dermatitis of hands and eyelids while working with penicillin in hospital wards. Eruption cleared during days off duty. Patch and intradermal testing with either "O" or G resulted in positive local reactions at site of test and a flare-up of lesions upon the hands and eyelids on several occasions, the last two years after the initial manifestation of contact dermatitis.

Only one case demonstrating the "id" phenomenon has been seen. In this individual (No. 5, Table III) response after administration of a large dose of "O" (400,000 units intramuscularly) was identical with the response to previous administration of G. Clinical syndrome after each agent was characterized by vesicular dermatitis involving hands, feet, axillae and groin.

Skin and Patch Testing. Skin and/or patch tests with 20,000 units penicillin "O" or G have been studied in seventeen individuals (two cases, Table I; ten cases, Table II; five cases, Table III). Skin testing with the materials did not aid in predicting the success of substitution of "O" for G in individuals suffering from a hypersensitivity reaction. Neither were skin and patch testing procedures practicable in confirming the presence of hypersensitiveness, suspected by history, to penicillin "O" or G; indeed, they might be considered misleading.

Twelve individuals with proven immediate or delayed anaphylactoid reactions were subsequently skin tested. Only five of the twelve had positive skin or patch tests. Of these five, two were definitely benefited by substitution therapy with disappearance of the initial allergy and its failure to recur in as severe a form upon readministration of penicillin G. Two others demonstrated such severe local reactions at the site of the skin tests that substitution of "O" for G was not attempted. The fifth individual was continued on therapy with "O" despite the persistence of a rash and this did not disappear until it was stopped. Seven individuals of the twelve tested had negative skin and patch tests to both penicillin G and "O" despite the previous presence of a known rash or urticaria to penicillin G. In all seven, substitution of "O" for G was totally successful. In six G was resubstituted for "O" without untoward event, but in one resubstitution of G was not possible. In no instance was the skin test positive for one agent and not the other.

TABLE III

OBSERVATIONS UPON PATIENTS DEMONSTRATING CONTACT DERMATITIS AND LOCAL REACTIONS TO PENICILLIN G

Case No.	Name	Age	Diagnosis	Initial Route and Exposure to Penicillin G	Reaction	Skin Test		Patch Test		Readministration of		Remarks
						O	G	O	G	O	G	
1	S. S.	26	.....	Habitual handling all forms penicillin G	Contact dermatitis	Pos.	Pos.	Not done	Not done	Acute flare up	Acute flare up	Nurse continues to have reactions, desensitization has to date been unsuccessful; see text
2	S. G.	25	.....	Penicillin ointment to face and aqueous G IM for 2 doses	Local and general urticaria	Pos.	Pos.	Pos.	Pos.	Not done	Not done	Handling of any penicillin preparations produces lesions on face; patch tests also flared this
3	L. S.	23	Septic sore throat	Oral penicillin 200,000 u 4 x daily for 4 doses	Edema of mucous membrane of mouth	Pos.	Pos.	Pos.	Pos.	See Remarks	Similar oral lesion	First administration of O—no reaction; 2nd administration of O—6 weeks later—local oral reaction but less severe than after G
4	S. A.	32	Asthma (dust and animal dander), nasal pharyngitis	Troches and ointment for 3 months; single injection of procaine G in oil	Urticaria	Neg.	Neg.	...	...	Not done	Not done	Penicillin ointment to nose daily for 3 months; urticaria for 8 months
5	N. S.	50	Asthma	Aqueous G, 400,000 units 5 doses	"id" (See text)	Neg.	Neg.	Neg.	Neg.	Same reaction	Same reaction	After 5 doses of G and 1 of O, asthma cleared; see text

Five patients demonstrating the other two less common forms of hypersensitivity were also skin or patch tested. (Table III.) One individual of the four with contact type of reaction had negative skin tests despite the presence of an overt reaction at the time of the test. The other three had positive skin tests. The single individual with, presumably, the "id" phenomenon had negative patch tests to both "O" and G.

COMMENTS

Results obtained in a study of the absorption, distribution, excretion and toxicity of penicillin "O" in healthy adults compare favorably with the pharmacologic properties of penicillin G in the human. This in itself suggests equal therapeutic efficiency in acutely ill patients with infections due to penicillin susceptible microorganisms. Penicillin "O" was used as the sole antimicrobial agent in fourteen patients acutely ill with a variety of infections, some severe. (Table I.) In all but one the infection was controlled as promptly and as completely as could

be expected with the use of penicillin G. In the one death reported penicillin could not have been expected to alter the course of the fulminating disease. With knowledge of its pharmacology in the human it is safe to say that "O" can be substituted for G unit for unit.

In eighteen additional patients (Table II) penicillin "O" was used in the course of treatment of moderately severe infections as a substitute for penicillin G in the presence of a delayed type of allergic reaction to the latter. Five patients (Tables I and II) also received penicillin "O" for prophylactic reasons. Three of these had developed a rash from administration of penicillin G and in each the prophylactic regimen with penicillin "O" was uneventfully continued.

From studies made upon individuals with the less common types of allergic manifestations to penicillin G (Table III) it is evident that penicillin "O" is not a total substitute for G in some circumstances. Indeed it appears that on certain predictable occasions there are cross immune reactions between "O" and G, particularly in instances of contact dermatitis and the "id" phenomenon.

It can be presumed from skin and patch testing observations that in the presence of a positive skin reaction to patch or intradermal testing, clinical hypersensitivity to penicillin exists, present or remote, but that the hypersensitiveness may very well be of no clinical importance. It is also apparent that an active allergic reaction may be present despite negative skin or patch tests.

Readministration of penicillin G to twelve patients treated with "O" during the one and one-half to fourteen days after subsidence of the skin reactions was possible without recurrence of the original hypersensitivity phenomena. This in no sense indicates that penicillin "O" aids the human host to desensitize himself to the antigen that produced the initial reaction. It only suggests that the induced reaction to penicillin G is, in most instances, temporary. It has been observed by many that desensitization occurs in most individuals exhibiting allergic reactions to all penicillin preparations without any procedure such as hyposensitization, and that the naturally occurring desensitization may be complete in a few days. Experience has also shown that continued administration of penicillin in the face of overt evidences of hypersensitivity is possible and that explosive, serious or acute anaphylactic accidents rarely occur under these circumstances. Nonetheless, there is always hesitation in continued exposure to the antigen. The place of penicillin "O" is quite clear cut in the majority but not all of these instances. When therapy cannot be discontinued in the patient with a serious infection because of a hypersensitivity reaction, substitution of penicillin "O" for G can be considered a safe and beneficial procedure. This is of very real advantage to both the patient and his physician.

Hypersensitivity phenomena to penicillin "O" occurred in this series in at least three instances. (Cases 4, 5 and 18, Table II.)

SUMMARY

Pharmacologic properties of allylmercaptomethyl penicillin (penicillin "O") have been described. A series of patients with a variety of acute infections who were treated continuously or intermittently with penicillin "O" have been described. From the data it is evident that penicillin "O" has similar pharmacologic and antibacterial activity to penicillin G in the human and that it can be substituted safely for the latter in therapy of even the most serious infections, unit for unit. It is a complete substitute in most individuals exhibiting delayed anaphylactoid reactions to penicillin G. In instances of contact dermatitis from penicillin G and in those manifesting the "id" phenomena, penicillin "O" virtually always fails to be a satisfactory substitute. These latter two types of hypersensitivity reactions to penicillin G are, of course, far less common than the delayed type. Thus there is no evidence that penicillin G and "O" are devoid of cross immune reactions. It is also likely that individuals can become sensitized to penicillin "O" but the frequency of this phenomenon cannot be stated.

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Allergy to Penicillin O and the Management of Penicillin Sensitivity*

SHEPPARD SIEGAL, M.D.
New York, New York

In introducing the new biosynthetic penicillin O the claim has been advanced that it represents a safe substitute for penicillin G in the majority of patients with penicillin sensitivity.[1,2] The advantages of having at hand another penicillin devoid of cross sensitization with penicillin G are obvious since allergy to penicillin is not uncommon and at times represents a hazard which may preclude use of this important antibiotic.

However, Risman and Boger[3] have published extensive observations on skin test findings with penicillins G, O and BT and conclude that cross sensitization is common. Their work and the five cases to be described indicate, therefore, that optimism concerning the usefulness of penicillin O in penicillin allergy should be somewhat tempered. These cases illustrate the occurrence of allergy to penicillin O as a result of sensitization by penicillin G.

The present report is based upon skin testing a small group of patients with a history of penicillin allergy. They have been tested with crystalline penicillin G and with penicillin O. The technic of the penicillin skin test is simple. A solution of penicillin in distilled water is employed containing 20,000 units per cc. For the immediate reaction 0.02 cc. is given intradermally, as in the usual small wheal for allergy testing, and the reading of wheal and flare is made at fifteen minutes. This reaction very often is negative. More commonly positive is the twenty-four to forty-eight hour delayed skin test elicited by the intradermal injection of 0.1 cc. of the test solution. A positive reaction consists in a definite area of erythema, with a diameter of 10 mm. or more, often with indurative edema.

In each case the skin test reactions of the two antibiotics have been compared; in Case v several such tests have been made. Skin test titrations and serial testing have not been attempted.

This report is not offered as a definitive study of the relationship of penicillin G and O allergy but rather as a warning to physicians that penicillin O may not be tolerated by the patient who is hypersensitive to penicillin G. In such cases it is important that the administration of penicillin O be carried out with caution and preferably only after preliminary skin testing. The limitations of skin testing as a measure of systemic sensitivity should, of course, be kept in mind.

CASE REPORTS

CASE I. M. W., a dentist, age twenty-seven, was given 300,000 units of aqueous procaine penicillin for three successive days beginning June 12, 1950, for acute sinusitis. Twelve days later he developed pruritus about the buttocks, generalized achiness and malaise and a temperature of 102.6°F. The pruritus became generalized and a diffuse urticarial eruption appeared. There was a large indurated erythematous area of the right buttock. Vesicular lesions of the third and fourth fingers of the right hand, edema of the feet and marked polyarthralgias were also noted. The patient responded well to salicylates and benadryl 100 mg. by mouth every four hours.

He had previously tolerated penicillin without difficulty in 1947 and again in January, 1950. He gave no personal history of allergy. However, on the maternal side there was a history of hay fever and asthma. He had had previous epidermatophytosis for ten years and had a trichophytid of the right hand a few years before.

On September 9th a skin test with aqueous crystalline penicillin G, 20,000 units per cc.,

* From the Medical Services of the Mt. Sinai Hospital, New York, N. Y.

gave a 4 plus immediate reaction with a huge wheal and extensive surrounding erythema. The twenty-four to forty-eight hour test was negative. On October 2nd the test was repeated with aqueous penicillin O in identical concentration. Again there was an intense immediate reaction identical with that elicited by penicillin G. It was evident that this patient was persistently and exquisitely sensitive to both penicillins G and O. He was advised to avoid therapy with either penicillin. It was suggested that retesting with both penicillins be deferred for a further two-month interval but unfortunately the patient was not available for further testing.

Case II. H. L., a fifty-five year old man, developed a positive skin reaction to crystalline penicillin G in the course of patch tests and intradermal injections of penicillin G when tested in 1945. In December, 1945, he had a forty-eight hour skin test reaction of 20 mm. diameter, with 2,000 units of crystalline G in 0.1 cc. saline. He was partially desensitized over a three-month period with from 80 to 20,000 units in ascending dosage subcutaneously. In April, 1946, the penicillin G skin test gave only a faint erythema. The trichophytin test in 1:30 dilution was 3 plus. After an interval of two and one-half years the patient was again skin-tested in December, 1949, with both penicillins G and O and found to give equal slight positive reactions measuring 12 mm. maximum diameter to both penicillins. This case indicates that even with mild degrees of penicillin G sensitivity cross sensitization to O may be demonstrable.

Case III. S. P., a thirty-seven year old male social worker, took penicillin tablets for two days in January, 1947, for an upper respiratory infection and one week later developed slight urticaria and pruritus. The pruritus continued for four months with gradual subsidence. An intradermal test with aqueous crystalline penicillin G, 2,000 units in 0.1 cc. of distilled water, yielded an intense 15 mm. erythema reaction of the delayed type which reached its height in one week, being only of moderate degree at the usual forty-eight hour reading. The immediate penicillin skin test and the trichophytin skin test were negative. Both personal and familial history were negative for allergy.

Subcutaneous desensitization was carried out with penicillin G in doses ranging from 100 to 50,000 units, nineteen injections being given in eight weeks. At this time the penicillin skin test became negative.

The patient was not seen again until January, 1951. He related that he had been well and without urticaria until March, 1950, when he was again given a single penicillin injection. Three months later he developed a three-week episode of mild migratory urticaria. He was again well thereafter. Intradermal tests with aqueous penicillins G and O, 2,000 units in 0.1 cc., gave positive skin reactions to both forms at forty-eight hours. The reaction to penicillin G was the stronger, reaching a maximum diameter of 25 mm. of moderately intense erythema. The penicillin O reaction was about half as strong, being 12 mm. of milder erythema.

In this patient penicillin G had induced sensitivity to both forms but the penicillin O cross sensitization was of milder degree than that to penicillin G itself. Further, while desensitization had been accomplished in terms of reversal of skin test two years previously, the test had reverted to positive, possibly as a result of re-exposure to penicillin.

Case IV. A. B., a man of forty-four, had two injections of penicillin, oil and beeswax in January, 1948, for an external nasal infection. After a ten-day interval he developed erythema and edema of the face, hands and feet without vesiculation. For two weeks thereafter he had an intense migratory pruritic macular erythema, which tapered off during a succeeding three-week period. He had previously been given crystalline penicillin in 1946 without incident. There was no personal or familial history of allergy.

On November 1, 1949, skin tests with aqueous crystalline penicillins G and O, 2,000 units in 0.1 cc., elicited forty-eight hour marked positive reactions of equal degree, with a maximum diameter of erythema about 35 mm. and an inner brighter zone of infiltrated erythema of 20 mm.

Because of the evident intense persistent sensitivity to both penicillins G and O an attempt at desensitization was made. During an eight-week period seventeen injections of penicillin G were given in ascending dosage from 100 to 100,000 units. At this time a skin test with penicillin G resulted in a reaction with onset at six hours which subsided rapidly. At forty-eight hours there was only a faintly visible erythema. This represented a very considerable reduction from the markedly positive skin test. In October, 1950, after an interval of nine months, the patient was found skin test negative to penicillin

O. This case illustrates not only sensitization to penicillin O as a result of penicillin G treatment but also successful though not quite complete desensitization to both penicillins G and O employing penicillin G alone.

Case v. I. R., a woman of fifty-two, had a respiratory infection in March, 1950. She was given a single procaine penicillin injection. Although one year previously she had tolerated penicillin treatment without reaction, on this occasion she developed intense generalized urticaria three weeks after the injection. This subsided after a few weeks but quickly recurred. Lesions then continued to appear daily until the patient was first seen on August 23rd after a period of six months. She complained not only of quarter-sized pruritic indurated red lesions scattered over the body surface, together with smaller scattered wheals, but also of typical angioedema distorting the face, lips and eyelids. There was no fever or joint pains. The sole history of allergy was of urticaria for two hours eighteen months previously. The family history was negative for allergy.

General physical examination was negative except for the skin lesions. There was a small wheal at the left wrist and a quarter-sized circumscribed circular plaque of indurative erythema over the anterior chest. The face appeared puffy and edematous. Urine analysis was negative; hemoglobin was 86 per cent; blood smear: normal differential count with 4 per cent eosinophils. Skin tests were made with crystalline penicillin G and with penicillin O, 2,000 units in 0.1 cc. At twenty-four hours there were strongly positive lesions which precisely duplicated the spontaneous plaque-like lesions of the patient's urticaria. These reached a maximum diameter of 35 mm. Immediate penicillin skin tests were negative. Trichophytin 1:30 skin test was negative.

In view of the clear-cut skin test reaction to penicillin there no longer existed any doubt that the six-month urticaria was actually due to persistent penicillin sensitivity. She had been previously treated symptomatically, with rigid dietary manipulation and with autohemotherapy to no avail. Desensitization was started August 24th with 50 units of aqueous penicillin O and continued twice weekly. The dosage increases were frequently limited by both local or mild general reactions indicating systemic sensitivity to penicillin O. Thus 40,000 units of penicillin O caused both local erythema and transitory swelling of the proximal metacarpophalangeal joints of two fingers. Desensitization with penicillin O was continued to a level of 200,000 units. At this time simultaneous skin tests with both forms of penicillin showed delayed positive reactions but almost twice as intensely to penicillin G. Further treatment reaching a dose level of 600,000 units of penicillin O was followed by skin testing which demonstrated a marked discrepancy in the size of the test reactions to penicillins G and O. The reaction to penicillin G showed a 20 mm. central zone of infiltrated bright erythema with a surrounding faint erythema of 30 mm. whereas that to penicillin O showed only faint erythema 15 mm. in diameter.

In view of the incomplete result with penicillin O further desensitization was continued with penicillin G. Dosage was increased up to 900,000 units of aqueous procaine penicillin and on another occasion to 750,000 units of aqueous penicillin G. At this time there was still slight itching at the injection site and of the scalp as well. Repetition of the skin tests with penicillins G and O at this time showed a further diminution in the reaction to penicillin G and a completely negative response to penicillin O.

This case of unusually prolonged urticaria and angioedema due to persistent penicillin allergy was promptly cured after desensitization was started. Phenergan was used at the onset in 6 to 12 mg. doses once or twice daily for symptomatic control and may well have contributed to the rapid subsidence of this stubborn urticaria. A tedious course of desensitizing injections was necessary to raise the patient's tolerance for the antibiotic to the point where she could tolerate full therapeutic doses. That this was achieved was shown by her lack of reaction to daily aqueous procaine penicillin injections given after a herniorraphy performed in March, 1951.

This case demonstrates not only the coincidence of penicillin G and O sensitivity as a result of treatment with the former but also the disappearance of the positive skin reaction to penicillin O while that to penicillin G persisted. Under desensitization with penicillin O* alone there was achieved a much more marked reduction of the penicillin O skin test while the effect upon the penicillin G test was slight.

* The author is indebted to the Upjohn Company for the penicillin O used in this study.

COMMENT

The five cases presented in this report illustrate cross sensitization to penicillin O due to penicillin G allergy. They cast doubt upon the assumption that in the majority of cases penicillin O can be safely administered to patients hypersensitive to penicillin G. This conclusion has been largely based upon the clinical observation that allergic manifestations due to penicillin G clear up when the therapy is changed to penicillin O. This is deceptive evidence because it is equally true that most such allergic reactions subside just as readily even though the patient continues to receive penicillin G itself. It should also be pointed out that many patients who have had initial reactions to penicillin G of the serum sickness-like type can tolerate subsequent courses of treatment without difficulty because they have not been persistently sensitized. By the same token, acceptance of penicillin O by such patients does not constitute satisfactory proof that penicillin O is a safe substitute for penicillin G in the persistently allergic patient. The patients of the present small series are instances of persistent penicillin G sensitivity and in each case there was a positive skin test to penicillin O as well. In Case v the penicillin O allergy was proved to be systemic in character when the patient reacted generally to the desensitizing injections of this penicillin.

It was hoped that substitution of the allylthiomethyl radical of penicillin O for the benzyl radical of penicillin G might so alter its antigenicity that it would be less of a sensitizing agent and would not show cross sensitization in cases of human penicillin allergy. Observations on guinea pig anaphlaxis[4] employing penicillins G, BT and O as antigens tended to support this concept. The newer biosynthetic penicillins were found not to sensitize guinea pigs whereas sensitization occurred for the most part with penicillin G. These investigators likewise found no evidence of cross sensitization to the new penicillins in guinea pigs injected with penicillin G. However, previous workers had found penicillin G a weak and variable anaphylactic antigen[5,6] but nonetheless it has proved to be an active sensitizer in human subjects.

Risman and Boger[3] have already demonstrated the occurrence of cross sensitization to penicillin O in human penicillin G allergy in a study of 224 patients. They employed the twenty-four to forty-eight hour skin test as an index of penicillin sensitivity. This test is a reliable guide to the existence of some degree of penicillin allergy although not necessarily associated with a systemic reaction to penicillin administration. Thirty-six patients gave a positive reaction to penicillin G and of these twenty-five were also sensitive to penicillin O. Thus approximately two of every three patients sensitized to penicillin G also became sensitive to penicillin O. Further analysis of their data shows that of twenty-one patients who had a known allergic reaction following treatment with penicillin G fourteen gave a positive skin test to penicillin G, of whom twelve reacted on test to penicillin O also. These are the patients who had had a reaction to penicillin and developed persistent sensitivity. The findings of these investigators suggest that only a small minority of such patients will be found insensitive to penicillin O. The five cases of the present report corroborate this conclusion.

It is therefore unsafe for the physician to assume that penicillin O can be safely administered to penicillin G sensitive patients. Suitable precautions must be taken to avoid allergic reactions. Preliminary skin tests with both penicillins G and O should be performed before using the drug. Positive reactions to both, particularly if strong and equal in intensity, would indicate great caution in the employment of either. In the exceptional case in which a negative skin test with penicillin O is found while that to penicillin G is positive, it is probable that penicillin O will be well tolerated. When, as in Case III, the reaction to penicillin O is distinctly less than that to penicillin G, employment of the newer penicillin might offer some advantage. Negative reactions to both do not exclude sensitivity and such patients must be given initial small exploratory ascending doses of either penicillin to determine whether penicillin allergy still persists.

How frequently penicillin O will be found to induce allergic reactions in persons never previously exposed to penicillin still remains an open question. This must await extensive clinical application of the newer penicillins. If they should prove less antigenic they might, assuming equivalent therapeutic value, displace penicillin G as the preparation of choice especially for atopic and other easily sensitized individuals.

Some fundamental facts pertaining to the proper allergic management of penicillin hyper-

sensitiveness might bear brief recapitulation at this time:

1. Penicillin treatment need not be discontinued because of the occurrence of a mild or moderate allergic reaction. Continuance of treatment can often be facilitated by the many symptomatic and anti-allergic remedies now available. This is of particular importance when a course of penicillin must be completed, as in Streptococcus viridans endocarditis.

2. Penicillin is tolerated without incident by the great majority of patients who give a history of a single previous allergic reaction to the drug. This is true even though the reaction has occurred only a few days or weeks previously.* A common error of present day practice is the assumption that the patient is persistently allergic to penicillin and must avoid the drug at all cost merely because of such a history. A history of repeated reactions is far more significant.

3. The penicillin skin test has a definite although limited area of usefulness. Just as with a positive skin test to ragweed, a positive reaction does not necessarily indicate clinical sensitivity. It does, however, indicate that an immunologic or allergic change has taken place in the patient. In such persons there is a greater likelihood of a clinical reaction to penicillin then in those with a negative skin test. A positive reaction does not preclude the possibility of administering the antibiotic but treatment should be given cautiously with preliminary smaller doses. The diagnosis of persistent penicillin allergy is therefore most accurately determined by a preliminary skin test and provocative test injections of the antibiotic in small ascending doses. On occasion a positive penicillin skin test serves as a useful diagnostic aid in obscure cases of urticaria (Case v) or of chronic dermatitis or eczema. A negative skin test does not exclude penicillin allergy.

The method of performing and reading the immediate and delayed penicillin skin tests has already been outlined. When an unusual degree of penicillin sensitivity is suspected, it is wise to make a preliminary test with a more dilute solution containing 2,000 units per cc. In contact dermatitis patch tests should be made prior to intradermal testing. All such tests can be as readily made with the new penicillins, such as penicillin O, as with penicillin G.

* This applies especially to the common urticaral or serum sickness-like reactions. The less frequent erythemato-vesicular form may be more prone to recurrence.

4. When the patient has been proved persistently allergic to penicillin, recourse will often be had to the sulfonamides or the newer antibiotics. However, desensitization to penicillin should be given serious consideration particularly when a penicillin-sensitive patient is scheduled for a major operative procedure or when for other reasons a course of penicillin treatment is contemplated. When the degree of sensitivity is such that desensitization might prove hazardous (Case i), it is best postponed or omitted altogether. Although often successful,[7,8] desensitization in some instances may be difficult (Case v) or even impossible. Patients who cannot be desensitized or who react violently to penicillin should be warned against future exposure to the drug.

5. The technic of desensitization requires the hypodermic injection of aqueous crystalline penicillin in ascending dosage two or three times weekly. The starting dose is often about 50 to 100 units. Increments are made as judged by the general or local reaction to previous injections and in the light of the intensity and type of the patient's past manifestations of penicillin allergy. Dosage should be increased to a point where full therapeutic levels can be tolerated. In cases with a positive skin test conversion to a negative test serves as a useful guide to successful desensitization.

SUMMARY AND CONCLUSIONS

1. Five cases are described which demonstrate the occurrence of allergy to penicillin O as a result of sensitization with penicillin G. In each instance positive skin tests to both forms of penicillin were obtained.

2. Penicillin O is not always a safe substitute for penicillin G in penicillin allergic patients. A review of the available evidence indicates that cross sensitization to penicillin O is the rule rather than the exception among those patients who are persistently allergic following a reaction to penicillin G.

3. It is therefore unsafe to administer penicillin O to patients who are actively allergic to penicillin G without suitable preliminary precautions. Nevertheless, it must be stressed that the majority of patients who give a history of an allergic reaction to penicillin do not remain allergic and can tolerate both penicillin G and penicillin O.

4. The allergic management of patients with penicillin sensitivity is reviewed. The distinction

between transitory and persistent sensitization to penicillin is emphasized, the role of the penicillin skin test is evaluated and the question of penicillin desensitization is explored, with a brief exposition of its technic.

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Review

Infectious Mononucleosis

A Study of 210 Sporadic Cases

JOHN E. STEVENS, M.D.,* EDWIN D. BAYRD, M.D. *and* FRANK J. HECK, M.D.

Fellow in Medicine, Mayo Foundation — Division of Medicine, Mayo Clinic

Rochester, Minnesota

INFECTIOUS mononucleosis, a disease of varied guise, frequently escapes detection. With an expanding armamentarium for the treatment of infectious diseases, both bacterial and viral, early accurate diagnosis once again becomes important in the selection of the proper therapeutic agent. It was with this in mind that the following study of 210 sporadic cases observed at the Mayo Clinic over a period of ten years, 1937 through 1946, was undertaken.

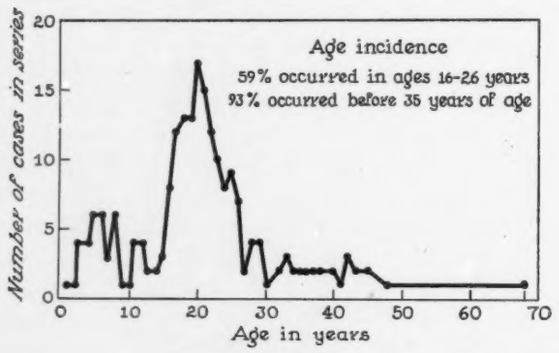

FIG. 1. Age incidence.

For convenience the history of infectious mononucleosis may be considered in four parts. The first phase was largely descriptive. Filatow in 1885 described an idiopathic adenitis occurring in children which was probably infectious mononucleosis. Pfeiffer[16,23] in 1889 described Drüsenfieber ("glandular fever") as a clinical entity which he considered a specific disease of the lymph glands in infants and children. Other names applied were acute idiopathic lymphadenitis,[21] monocytic angina,[1] acute benign lymphoblastosis[2] and acute lymphadenosis.[9]

Sprunt and Evans in 1920 drew attention to the characteristic changes in the peripheral blood and proposed the name infectious mononucleosis, initiating the second period. Earlier observers had previously noted leukocytosis,[13] large lymphocytes[26] and an increase of mononuclear elements in the blood.[3,4,18] Longcope described the morphologic abnormalities in the blood and the histology of excised lymph nodes. The studies of Downey and McKinlay in 1923 on the morphology of the lymphocytes found in this disease concluded this phase of investigation and left little of importance to be added from a hematologic point of view.

The third phase of interest in infectious mononucleosis was introduced in 1932 when Paul and Bunnell first described their now well known sheep cell heterophil agglutination test.

The present phase may be considered to date from 1944 when Ziegler published the first report of necropsy in a case of infectious mononucleosis in which death was due to rupture of the spleen. Other reports followed[7,15,19,24,27] and showed that the disease process was generalized and affected many organs. The general pathologic aspects have been well summarized by Custer and Smith. Hovde and Sundberg have recently described focal granulomas in the bone marrow.

STUDY OF 210 SPORADIC CASES ENCOUNTERED AT THE MAYO CLINIC

Cases in which either the blood smear or the heterophil agglutination test was positive for infectious mononucleosis and the clinical findings were compatible with this diagnosis were accepted for this study. A blood smear was considered to be positive when at least 50 per cent of the leukocytes were lymphocytes and a significant number of the lymphocytes were of the abnormal infectious mononucleosis type described by Downey. Heterophil agglutination was considered to be positive when the patient's

* Present address Richmond, Va.

serum contained agglutinins for sheep red blood cells in a titer of 1:224.

Age, Sex, and Seasonal and Occupational Variation. The age of the patients ranged from nine months to sixty-eight years (Fig. 1) with 59 per cent occurring between the ages of sixteen and twenty-six years. Ninety-three per cent of the patients were less than thirty-five years of age. Fifty-seven per cent were females. (Fig. 2.) No racial predilection was noted. The highest incidence (31 per cent) was in the summer months. (Fig. 2.) This is contrary to the experience of others and may be related to the increased patient load at the clinic in those months. Patients of no one particular occupation (Fig. 2) were especially affected.

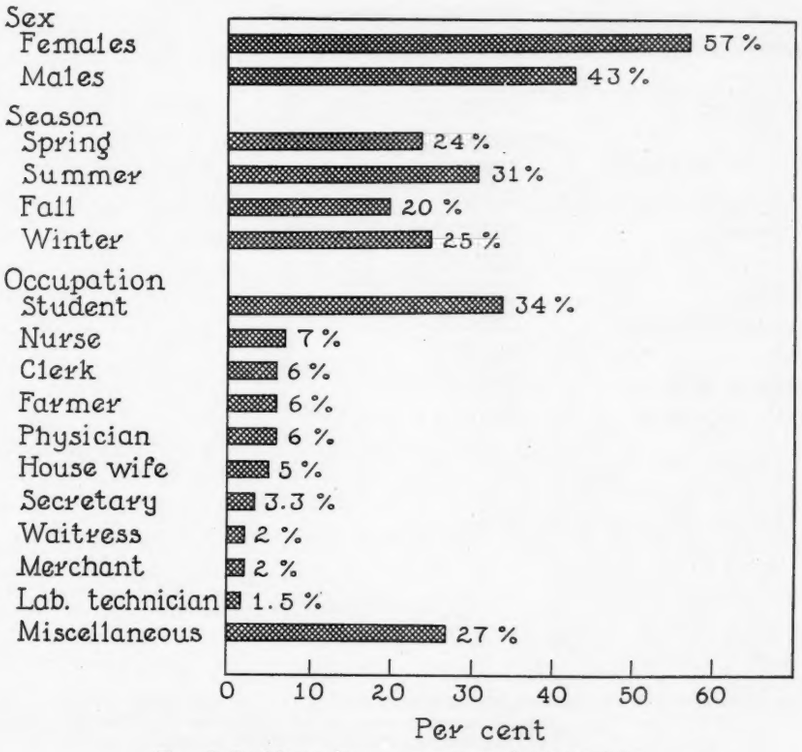

FIG. 2. Incidence by sex, season and occupation.

Epidemiology. Information relative to epidemiology was limited, the incubation period could not be estimated. Eight patients (4 per cent) of the total group were found to have had dental sepsis or recent dental extraction. Five of these patients volunteered the information that the symptoms of which they complained on admission had their onset from three to twenty-one days following the extraction of an infected tooth. Three other patients when seen on admission were found to have one or more periapical abscesses. One of these was seen at the clinic because of the third relapse of infectious mononucleosis and stated that for the preceding five months one of his principal complaints had been toothache. Examination revealed a periapical abscess that had ruptured. Subsequent to or coincidental with extraction of the offending tooth an uneventful recovery without further relapse occurred.

Infectious mononucleosis developed during convalescence from a surgical procedure in eight cases.

Clinical Manifestations and Complications. The protean nature of infectious mononucleosis may be appreciated by noting the most frequent chief complaints (Fig. 3) and the more common clinical findings. (Fig. 4.) The significance of this is even more apparent when a review is made of the numerous diagnoses on admission. (Fig. 5.)

The onset was insidious in 58.5 per cent while in the remainder it was acute. (Fig. 3.) Rarely symptoms were absent and the disease was discovered in the course of a routine physical examination. In contrast, others were prostrate with high fever and their disease ran a protracted course.

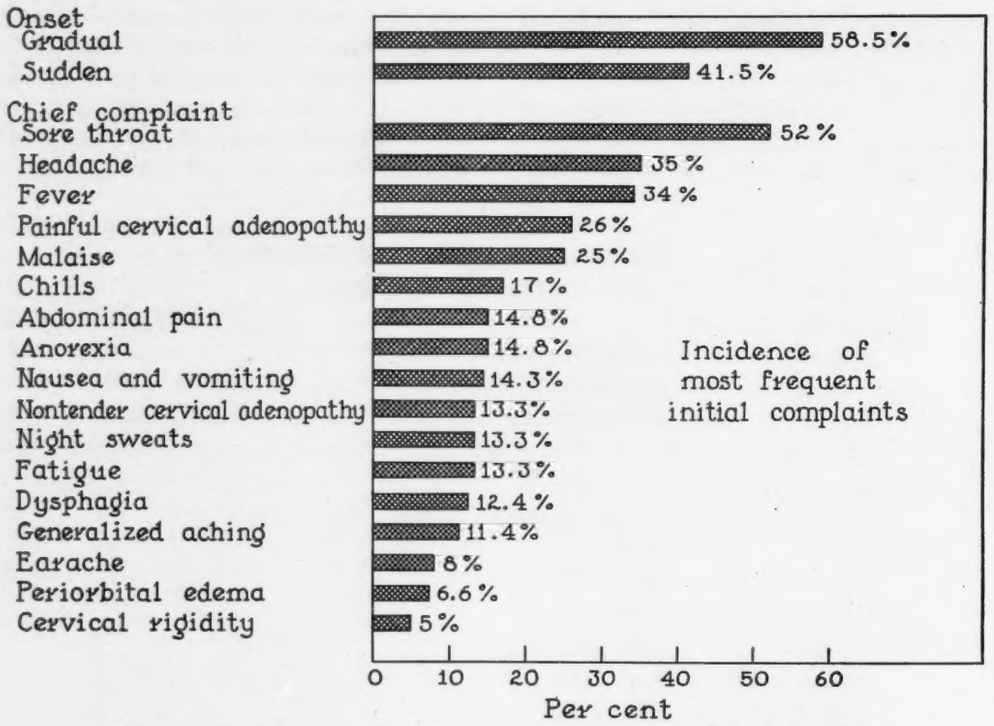

FIG. 3. Type of onset and most frequent chief complaints in this series.

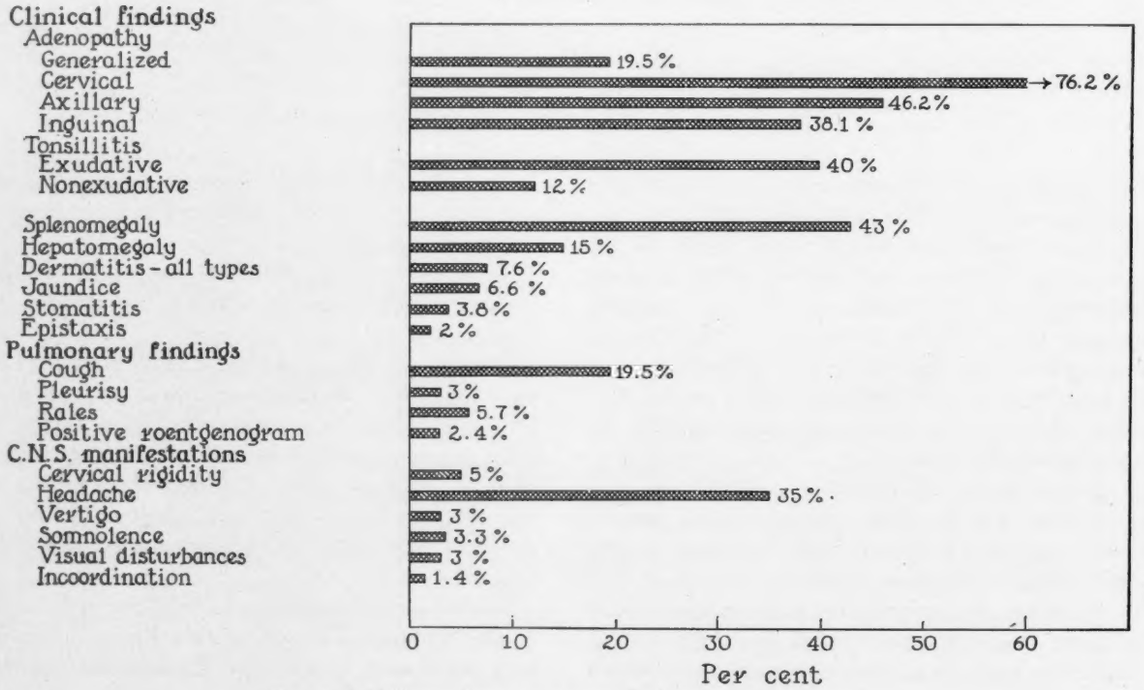

FIG. 4. Incidence of more common findings in 210 patients.

Respiratory manifestations: Sore throat was the outstanding complaint of the patients (52 per cent) in this series. (Fig. 3.) Exudative or membranous lesions on the tonsils or pharynx were noted in 40 per cent. (Fig. 4.) Lymphoid hyperplasia and marked edema caused dysphagia in 12 per cent. Stomatitis was observed in 3.8 per cent of the cases. In twenty-three cases beta hemolytic streptococci were cultured from the pharynx. In one instance peritonsillar abscess had developed as a sequel to severe tonsillitis. Another patient had an abscess in the posterior nasopharyngeal vestibule.

Roentgenographic evidence of pulmonary involvement was found in only 2 per cent of the patients in this series although cough, pleuritic pain and rales found on auscultation were noted in varying degree. (Fig. 4.)

Chills ushered in the disease in 17 per cent of the cases, rhinitis in 5 per cent and marked dyspnea in another 5 per cent.

Abdominal manifestations: Splenomegaly was detected in 43 per cent of the series (Fig. 4) and was encountered between the tenth and twenty-first days in 62 per cent of those in which it was present. Enlargement of the spleen was still present four months after the acute stage of the disease had subsided in two cases and in one case it was still palpable eight months after the acute stage had subsided.

Hepatomegaly was found in 15 per cent of the cases and was discovered by the fourteenth day of the disease in about half of these cases. As with splenic enlargement hepatomegaly might persist for weeks or months. Jaundice appeared in only fourteen of our patients, 6.6 per cent.

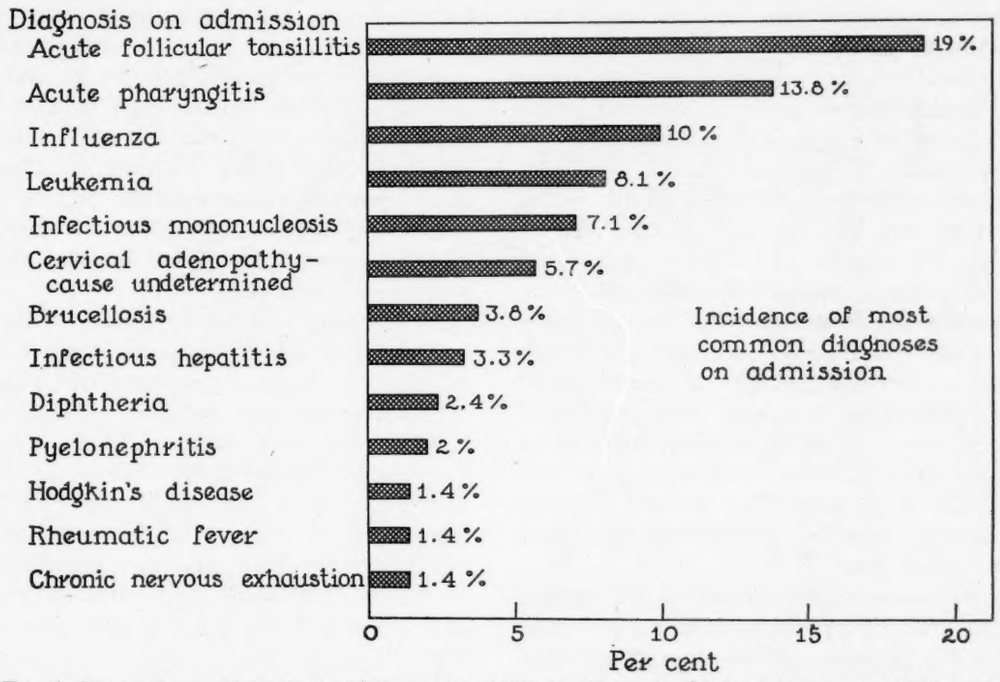

FIG. 5. Diagnosis on admission in 210 cases in which the diagnosis of infectious mononucleosis was made later.

Abdominal pain was the presenting complaint in 14.8 per cent. (Fig. 3.) In two cases it was of such severity that surgical exploration was performed as an emergency measure. In both cases the only significant finding was mesenteric adenitis. Anorexia, nausea and vomiting occurred with approximately equal frequency. Diarrhea was noted in seven cases. In two of these cases stools contained blood.

Neuromuscular manifestations: Myalgia, bachache and generalized aching was a common distressing, early complaint. About 35 per cent complained of headache, especially at the onset. Cervical rigidity was found in 5 per cent while somnolence, often prodromal, vertigo and visual disturbances, such as blurring, diplopia and scotomas each were noted in approximately 3 per cent. (Fig. 4.)

Mania was observed in one case in which the

clinical appearance was that of meningo-encephalitis while in another delirium was marked.

Vascular and lymphatic manifestations: Epistaxis occurred in 2 per cent of our patients. The bleeding varied from mild to severe and was recurrent over a period of a month in one case. Hematuria either gross or microscopic was observed in 13 per cent.

Demonstrable lymphadenopathy is not a *sine qua non* of this disease; however, cervical adenopathy was observed in 76.2 per cent and was the most consistent physical finding. (Fig. 4.) Generalized enlargement of the lymph nodes occurred in only 19.5 per cent of cases. Lymphadenopathy in order of frequency was noted as follows: cervical, axillary and inguinal.

Cutaneous manifestations: In sixteen cases (7.6 per cent) skin lesions developed. They were described as maculopapular in three cases, polymorphous (appearance of erythema multiforme) in three, urticarial in three, macular in two, morbilliform in two, hemorrhagic in two and nodular in one case. There seemed to be no correlation between the appearance of a rash and the clinical course.

Ocular manifestations: Edema of the eyelids and periorbital tissues was noted in 6.6 per cent. (Fig. 3.) Photophobia occurred in 4 per cent and, like burning and lacrimation seen in 2 per cent, it was an early manifestation. Conjunctivitis was observed in only two cases and uveitis in one.

Relapses and recurrences: In ten cases a relapse occurred within a period of four months. A relapse was considered to be reactivation of the disease before all manifestations of the preceding attack had disappeared. An increase in jaundice was the principal indication of relapse in three cases. Tonsillitis was the chief manifestation of relapse in four. Four months was the longest interval between attacks; the average interval was twenty-one to twenty-five days.

Recurrence was used to denote a second attack of infectious mononucleosis following an interval during which the patient was subjectively and objectively free of manifestations of the disease. Only two patients in this study could be classified as having had a recurrence. In both instances eight months elapsed between attacks.

Laboratory findings: In 97 per cent of cases smears of the peripheral blood showed a significant increase in atypical lymphocytes of infectious mononucleosis. In 55 per cent such a picture had appeared by the eighth day of the disease; the average day of appearance for all cases was the fourteenth day, with the time of their occurrence ranging from the first day to the eighth month of the disease. Serial smears were not made in each case but it is possible that, had this been done, the characteristic changes in the lymphocytes would have been exhibited in all.

Shift of the neutrophils to the left and granulocytic immaturity to the early myelocyte were encountered occasionally but toxic changes in the neutrophils were uncommon. The lowest total leukocyte count was 600 per cu. mm. of blood; the highest was 42,000. In general, the higher counts were noted among the more severely ill patients.

In Figure 6 the total number of leukocytes and the percentage of lymphocytes are noted in relation to the duration of the disease. No consistent pattern of rise and fall was detected.

Agranulocytosis occurred in one case. This patient, a physician sixty-five years old, was observed to have 7,600 leukocytes with 96.5 per cent lymphocytes and 3.5 per cent monocytes on the twenty-third day after admission. Four days later neutrophils reappeared in the peripheral blood and the subsequent course was uneventful.

While one expects to see lymphocytosis at some stage in the disease, it may not occur early. (Fig. 6.) Three patients, for example, had less than 20 per cent lymphocytes in the peripheral blood at the first determination. The average was between 65 and 70 per cent; two had more than 95 per cent lymphocytes. Usually a reversion to more nearly normal values began after the peak was reached, generally by the fifteenth day, and was completed by the twenty-fifth to the thirtieth day. Lymphocytosis persisting for months was not uncommon, however.

Of eight cases in which tests for liver function were performed all showed impaired function. This has been noted by others.[5,8,10,12,20]

Bile was found in the urine of seven patients; glycosuria was found in thirteen. Hematuria was seen in 13 per cent.

Agglutination of sheep's erythrocytes (heterophil agglutination) was noted in significant titer (1:224) in 91 per cent of 142 cases in which this test was performed. The highest titer encountered was 1:7,168. Most of the elevated heterophil agglutination titers were obtained after the

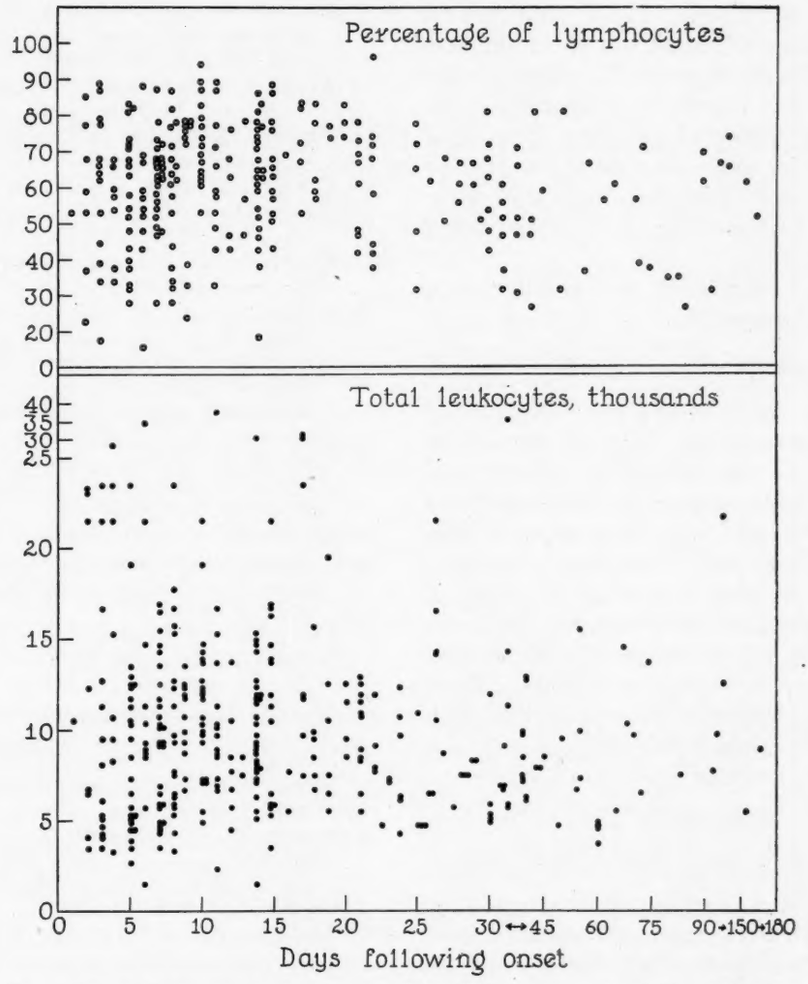

FIG. 6. Total leukocytes and per cent of lymphocytes on various days following onset of illness.

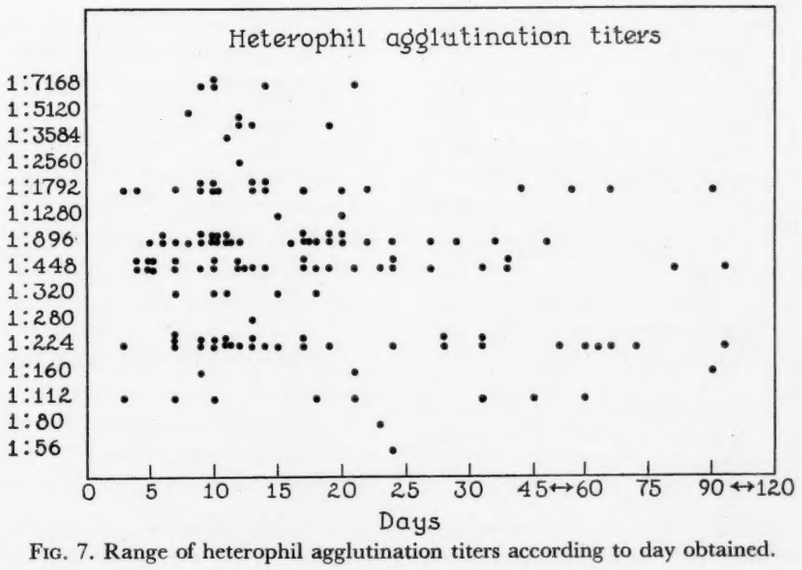

FIG. 7. Range of heterophil agglutination titers according to day obtained.

seventh day of the disease (Fig. 7) and in 51.4 per cent of cases a significant elevation was demonstrated by the fifteenth day. Blood withdrawn early in the disease was frequently negative. The titer remained elevated (Fig. 7) in some instances for more than three months. At other times elevations in titer were temporary and without frequent determinations might escape detection.

Four patients temporarily had positive serologic reactions for syphilis.

SUMMARY AND CONCLUSIONS

A study was made of 210 sporadic cases of infectious mononucleosis seen at the Mayo Clinic from 1937 through 1946. The clinical findings suggesting infectious mononucleosis are too indefinite and diverse to serve as final criteria on which to base a diagnosis. Reliance, therefore, must be placed on the observation of increased number of lymphocytes, with an appreciable number of leukocytoid forms and a significant titer of heterophil antibody. However, familiarity with the disease's varied cloak should guide the thoughtful clinician past the obstacles which beset the unwary.

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Seminars on Arteriosclerosis

Lipid Metabolism and Atherosclerosis*

R. Gordon Gould, ph.d.
Chicago, Illinois

Previous to the present series[1] of articles on arteriosclerosis, two excellent reviews in this Journal[2,3] covered briefly the principal points of cholesterol metabolism as related to atherosclerosis. The purpose of the present article is to discuss in somewhat greater detail certain aspects of lipid metabolism bearing on atheromatosis, emphasizing recent work on the origin of the plasma lipids, factors affecting endogenous lipid synthesis, relationships between plasma and tissue lipids and similar studies made possible by the use of radioactive isotopes.

While there is now fairly general agreement that a disordered lipid metabolism plays a role in arteriosclerosis, the nature of the disorder and its relationship to the disease appears more subtle and more complex than is implied by the concept merely of an elevated serum cholesterol level as etiologic agent. Gofman and associates[4] have recently shown that cholesterol-containing plasma lipoproteins of abnormal types are usually present when the total cholesterol level is very high in both humans and rabbits and that they may be present in humans even with so-called normal levels; furthermore, there appears to be a high degree of correlation between proved coronary artery disease and the presence of abnormal lipoproteins. This approach will very possibly clarify the long history of disagreement between different workers on whether or not atherosclerosis is correlated with an increased serum cholesterol, and will focus attention on the mechanisms responsible for the production of both normal and abnormal plasma lipoproteins.

From the clinical point of view the *distribution* of atheromatous plaques has an importance equal to that of their occurrence, for a relatively small total amount of atheromatosis, concentrated in a coronary artery may cause coronary occlusion whereas even large amounts spread diffusely throughout the aorta may pass unnoticed and without clinical manifestations for many years. Elucidation of the factors responsible for the variable distribution of plaques—the vagaries of arteriosclerosis, as Wolbach has called them—is an interesting but an extremely difficult problem to attack. Local factors such as stress on or damage to the vessel wall may be involved in addition to general metabolic and systemic factors. In view of the relative freedom from atherosclerosis shown by almost all mammalian species other than the human, the approach of greatest importance and promise would appear to be investigation of why atheromatous plaques form anywhere in the vascular system, rather than to attempt to determine why they are most prevalent in the coronaries of one person, the cerebral arteries of another and the aorta of a third.

In this country virtually every adult shows some degree of atheromatosis of the aorta and, indeed, even in young children it is by no means rare.† For this reason the tendency to assume that normal human adults with no demonstrable signs of vascular disease are free from atheromatosis and thus differ qualitatively from patients with coronary occlusion or other definite evidence of atherosclerosis is generally incorrect and has led to a good deal of confusion. Since the population as a whole must be considered to have atheromatosis, according to the findings of the pathologists, there is no control

† Lande and Sperry,[5] in a study of the aortas of eighty-nine individuals over thirty years old who died from trauma or alcoholism and without evidence at autopsy of disease other than atherosclerosis, found only three without macroscopically evident atheromatous lesions. Peck, McGill and Holman[6] in a series of 300 consecutive autopsies reported that there was no person beyond the age of seven years without at least one crop of early atherosclerotic lesions in the aorta.

* From the Rush Department of Biochemistry, The Presbyterian Hospital of the City of Chicago, affiliated with the University of Illinois College of Medicine. Aided by a grant from the Otho S. A. Sprague Memorial Institute and by a contract with the Atomic Energy Commission.

group and comparisons of patients and "normals" are actually comparisons between different degrees of severity and different anatomic locations of atheromatous lesions. From the standpoint of lipid metabolism, the real problem is the widespread if not universal chronic, symptomless disease of early atheromatosis. How is this related to the metabolism of cholesterol and other lipids, and how can the formation of atheromatous plaques be prevented?

Of all the animals examined, birds most closely resemble humans in developing atheromatosis spontaneously. In the chicken, for example, the disease appears at about six months of age and has been reported to have an incidence of about 45 per cent in both hens and roosters over one year old.[7] This disease and the factors affecting its development have been studied particularly by Katz and Dauber and their associates,[71] and by Chaikoff and associates.[8]

It has, of course, been well known for many years that cholesterol feeding alone will produce hypercholesterolemia and eventually marked atheromatosis in the rabbit; the guinea pig, chicken and duck have more recently been found to behave similarly. Even the dog may be made atherosclerotic by depressing thyroid function with thiouracil while cholesterol is being fed.[70] Although the doses fed and the plasma levels reached are both extremely high compared with those in patients, that is probably due not so much to necessity as to the impatience of the investigator who wishes to produce the disease experimentally in a matter of weeks rather than the years it takes to develop in the human. In man the influence of the cholesterol content of the diet on the gradual development of atheromatosis in the general population cannot be evaluated at present; but, as has been pointed out by Gubner and Ungerleider,[2] there is evidence suggesting that in this country the population as a whole exhibits some degree of hypercholesterolemia as compared with population groups in certain other countries. Plasma cholesterol values in the Orient usually range around 150 mg. per cent and in North Chinese coolies are often around 100 mg. per cent according to Snapper.[9] This has been attributed to diets low in animal protein and therefore in cholesterol, but it should be mentioned that they are also low in fats of all kinds and probably in total calories as well. In comparison with lower animals, plasma levels of Occidentals are conspicuously high; this is particularly interesting in view of the remarkably small amount of variation between species in the cholesterol concentrations in corresponding cellular tissues, as illustrated for erythrocytes in Table 1. Average plasma levels vary from 32 in the guinea pig to about 150 in the dog, with the human "normal" average given as 190 to 210[5,10] but values as high as 300 or even 350 are often considered as being in the "normal" range. Erythrocyte levels are no higher in humans than in other species, varying only between about 100 and 150 mg. per 100 ml.;[11,12] and other tissues such as liver, spleen, heart, skeletal muscle, etc., show a similar constancy although visceral organs have higher levels than erythrocytes or skeletal muscle. Whole body cholesterol, in those animals in which direct determinations have been made, also shows a relatively constant concentration in each species, being somewhat higher for the smaller animals; if calculated in terms of body surface area rather than weight, it would be more nearly the same for all species. In the human the total body content may be estimated from figures for individual tissues[13] as being about 130 mg. per 100 gm. of body weight, with 29 per cent in brain and 6 per cent in blood.

TABLE 1

CHOLESTEROL CONCENTRATIONS IN VARIOUS SPECIES IN MG. PER 100 ML. OR 100 GM. TISSUE

	Plasma	Red Cell	Whole Body
Mouse	...	...	300
Rat	52	...	200
Guinea pig	32	100	...
Rabbit	50	120	120
Cat	93	...	150
Chicken	100	135	...
Dog	150	140	...
Sheep	70	145	...
Hog	120	120	...
Cow	110	110	...
Horse	83	122	...
Human	200	140	...

The reasons for the high plasma levels commonly found in humans is a matter of dispute and is one of the key questions in the whole field. Both dietary and endogenous factors have been implicated. There is no question that the plasma cholesterol level is regulated by an efficient homeostatic mechanism since for a given individual it shows a narrow range, much

narrower than that for a large group. It may, however, be influenced to a certain extent by a number of factors, including diet (both the cholesterol and total fat content), age,[14,15] thyroid status,[16] diabetes; and to a very large extent by lipoid nephrosis, hypercholesterolemic familial xanthomatosis[17] and severe hepatic insufficiency, as for example in acute yellow atrophy. It shows a remarkable resistance to the ingestion of huge amounts of cholesterol over a short period of time; but, according to most investigators,[18,19] if cholesterol is fed in an easily absorbable form, for example as egg yolk, and the feeding is continued long enough, some increase in blood level will be obtained. Whether this is due entirely to the cholesterol in the diet or to the total lipid content is not entirely clear. Little is known about physiologic functions of cholesterol but the fact that the plasma level never falls very low regardless of diet, except in terminal states, is indicative of its importance to the organism.

Although the relative constancy of both phospholipids and cholesterol (and of their ratio) have been known since Mayer and Schaeffer's[11] and Terroine's[20] studies, until recently relatively little attention was paid to any plasma lipids other than cholesterol in relation to arteriosclerosis. Ahrens and Kunkel[21] pointed out that if the amount of phospholipid in plasma is 30 per cent or more of the total lipid, the plasma will be clear and appear non-lipemic even at total lipid levels as high as 3,000 mg. per cent; but if it is less than 30 per cent, the plasma will be cloudy and appear lipemic even at fairly low levels. In biliary cirrhosis both phospholipid and cholesterol are elevated, the plasma is clear and there may be very little atherosclerosis; whereas in other hypercholestemic states the plasma is usually cloudy and atherosclerosis is more prevalent. A number of investigators have suggested that the ratio between cholesterol and phospholipid in plasma is more important than the total cholesterol level in determining whether or not atheromatous changes will take place. The levels of the three principal groups of lipids in plasma do not usually change independently of each other, and the metabolism of cholesterol is so closely tied up with that of phospholipids and neutral fats that it is only by a consideration of lipid metabolism in general that we can hope to arrive at an understanding of the complexities of arteriosclerosis. Since only a few aspects of this large field can be mentioned here, reference is made to a number of recent discussions on different phases of lipid metabolism, including Peters and Van Slyke's comprehensive review[10] of lipid metabolism with references to practically all the literature up to 1946, Thannhauser's review on serum lipids in diagnosis,[17] Weinhouse's review on plasma cholesterol,[22] Page's discussion of arteriosclerosis and lipid metabolism,[23] Hirsch and Weinhouse's review on the chemical changes in arteries in aging and in arteriosclerosis,[24] Chaikoff and Zilversmit's[25] and Hevesy's[26] reviews of phospholipid metabolism as studied with radioactive phosphorus, Burr's review of the significance of essential fatty acids,[27] Cook's review of cholesterol metabolism,[13] Bloor's book on the chemistry and metabolism of lipids[12] and an excellent symposium on lipoproteins[39] held by the Faraday Society in 1949.

NATURE OF TISSUE LIPIDS

It has been customary to describe the properties of the lipids as they are obtained by extraction with organic solvents after denaturation of all tissue proteins rather than as they occur in tissue. For example, the various phospholipids are classified partly on the basis of differing solubilities in organic solvents although as they exist in plasma none of them are soluble in any organic solvent.

As long ago as 1931 Sorensen remarked that "the perfect clearness of such liquids as serum and plasma, in spite of their contents of lipoids, is explicable only by assuming linkage between lecithin and sterols on the one hand and the protein on the other."[28] Although neutral fat occurs in plasma in finely emulsified form, the fat droplets being stabilized by a protein film, it is probable that in normal plasma and in tissues all other lipids occur only in combination with proteins as lipoproteins. The properties of the lipids in lipoproteins are entirely different from those of free lipids; not only may lipoproteins be very soluble in water in spite of consisting of 75 per cent of water-insoluble lipids, but the chemical reactivities are undoubtedly very different also. The technical difficulties in their separation from other proteins and from each other are so formidable that only in the simplest case, plasma, are general methods now available for the isolation of lipoproteins under sufficiently gentle conditions to preclude any denaturation. A pioneer in this field was Macheboeuf who in

1928 recognized the existence of lipoproteins as entities, and isolated a water-soluble protein from horse serum which contained 40 per cent lipid and behaved as a homogeneous substance.[29] Cohn's group[30] has developed chemical methods for the separation of the two lipoproteins in normal human plasma identified by electrophoresis, the β_1 globulin and the α_1 globulin. The more abundant of the two, the β_1 lipoprotein, has been purified and characterized by Oncley, Gurd and Melin[31] and some of the data are shown in Table II. Kunkel[32] has proposed a simple procedure for the precipitation of all the lipoproteins in plasma in undenatured form. The ultracentrifugal method developed by Gofman is being applied widely for the analysis and separation of lipoproteins both in normal and pathologic sera, as will be discussed in a later article in this series. Although classical electrophoresis methods are not ideal for isolation of lipoproteins in pure form, the new method of filter paper electrophoresis[33] shows promise.

TABLE II
COMPOSITION OF THE β_1-LIPOPROTEIN FROM NORMAL HUMAN PLASMA[31]

	Gm. per 100 Gm. Lipoprotein	Moles per Mole Lipoprotein
Cholesterol, total	47.4	1040
Free	8.3	280
Esters	39.1	760
Phospholipids	29.3	500
Protein	23.0	1
C/PL ratio	1.05	2.1

Molecular weight estimated to be 1,300,000. The phospholipid fraction was about 85 per cent or more lecithins.

Liver contains lipids in two forms, (1) fat droplets easily identifiable histologically as lipid and containing primarily neutral fat in a free state, and (2) lipid in a form which is not evident histologically, containing cholesterol, phospholipid and some neutral fat probably all as lipoproteins. Although molecularly dispersed, these lipoproteins are not necessarily all water-soluble. In fact, fractionation of liver homogenate by differential high speed centrifugation has shown the lipids to be associated with some of the particulate elements of the cell. Mitochondria contain about 25 per cent and microsomes about 40 per cent lipid.[34] Lipids are also present in the limiting membranes of cells, mitochondria and nuclei, and in the nucleolus.

Special methods have been developed for the purification of particular biologically active lipoproteins from tissues, such as for example the thromboplastic protein from lung that Chargaff has isolated.[35]

There is, however, practically no literature on the metabolism of lipoproteins and indeed very little on their nature, but an immense amount of work has been done on the metabolism of the three principal types of lipids, neutral fat, cholesterol and phospholipids. The remainder of this article will be concerned with some of this work so a brief description of the chemical nature of lipids is given here.

Neutral Fats. These consist of mixtures of triglycerides of various fatty acids, saturated and unsaturated. They occur only in two types of cells: in liver cells and in special fat cells located in fat depots and interstitially in all tissues except the central nervous system. They contain less highly unsaturated fatty acids than phospholipids or cholesterol esters and can readily be synthesized in the body from carbohydrate or protein. The function of neutral fat is primarily that of energy storage and it therefore may vary more in amount than any other body constituent—from about 10 to 40 per cent of body weight, whereas other lipids are practically constant in amount.

Sterols. Cholesterol (Fig. 1, 1) is the chief but not the only member of the sterol group found in animals. It occurs in all body tissues and appears to be one of the essential constituents of all mammalian cells. Cholesterol or other sterols occur in lower forms of animal life down to and including sponges and possibly protozoa. No plants contain cholesterol but they all contain closely related sterols, the lower forms, ergosterol, and the higher, sitosterols, and in some cases stigmasterol. Unlike the vitamin B group, sterols are not essential for any form of life since many bacteria contain none; this suggests that their function may be in regulation of metabolic processes rather than as obligatory participants.

Cholesterol occurs partly in "free" form, i.e., with the hydroxyl group unesterified and therefore precipitable by digitonin, and partly esterified with fatty acids. In the adrenal 90 per cent of the cholesterol is esterified, in the plasma 67 to 72 per cent, but in most other tissues the ester makes up a small fraction of the total. In

liver it varies considerably with the species, being 20 per cent in man,[17] 50 per cent in rabbit and only about 5 per cent in dogs. Few data are available on whether there is a correlation between cholesterol esters in liver and atheromatosis in man. This might be of interest to investigate since it is known that cholesterol esters increase in the livers of animals fed cholesterol or made hypothyroid. Also, it has repeatedly been emphasized since the first investigations of Schoenheimer[41] that it is primarily cholesterol esters which accumulate in the arteries in atherosclerosis. It should be emphasized that normally cholesterol never occurs free in the sense of being uncombined but is always protein-bound, whether free or ester.

Two other sterols are present in small amounts in all tissues and a third in feces. Dihydrocholesterol (Fig. 1, II) is a constant companion of cholesterol to the extent of about 1 to 2 per cent in all tissues. Although impossible to separate from cholesterol by ordinary methods of recrystallization or digitonide formation, this saturated sterol was found by Schoenheimer[41] to differ from cholesterol in being unabsorbable from the intestine. A mixture of the two is excreted in bile and in intestinal juice but only cholesterol is reabsorbed, as shown by analysis of thoracic duct lymph. This provides a possible method for the excretion of unwanted excessive amounts of cholesterol but it is not known to what extent the body is able to utilize this mechanism.

The precursor of vitamin D_3, 7-dehydrocholesterol (Fig. 1, III), is found in small amount (0.1 to 2 per cent) in the cholesterol of all tissues but is particularly abundant in intestinal mucosa and skin. Scott et al.[36] have recently shown that the guinea pig intestinal mucosa is able to convert dietary cholesterol into the 7-dehydro form, which can then presumably be transported to the skin. The conversion into vitamin D_3, a non-steroid compound (Fig. 1, IV), by cleavage of the second ring in ultraviolet light is actually a breakdown of the steroid ring system of cholesterol. There is, however, no evidence that this type of breakdown is quantitatively important in cholesterol disposal.

A second saturated derivative of cholesterol, coprosterol (Fig. 1, VI), differs from dihydrocholesterol in the stereo-isomerism at the C_5 position. It has never been isolated from animal tissues but is normally present in feces and is formed in the colon presumably through the action of bacteria. A cerebroside, phrenosin, originally isolated from brain but present in small amounts in most animal tissues, is necessary for the conversion of dietary cholesterol to coprosterol. This important finding by Rosenheim and Webster[37] explains why infants on an

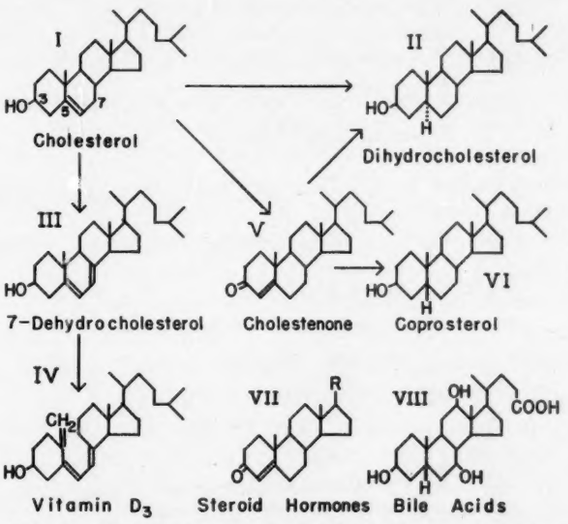
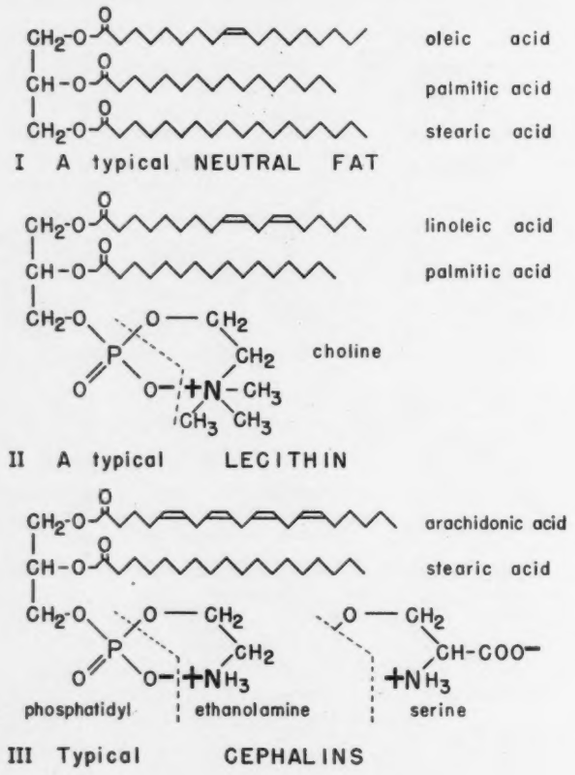


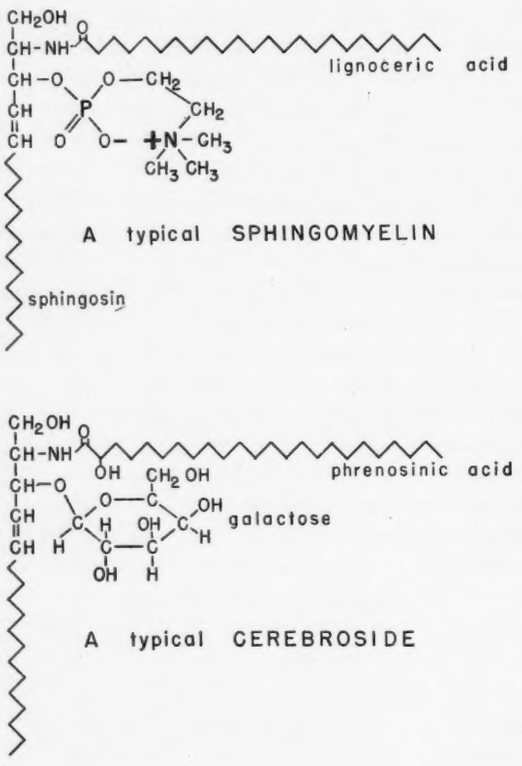
FIG. 1. Structural formulas of cholesterol and some related compounds. Cholesterol may be reduced directly to dihydrocholesterol and indirectly through cholestenone to either coprosterol or dihydrocholesterol. Several different types of oxidation are possible, including oxidative cleavage of the side chain to give the bile acids and steriod hormones. Note that the bile acids have the steric configuration at C_5 of coprosterol, rather than that of dihydrocholesterol.

exclusive milk diet and animals on a meat-free diet excrete cholesterol in the feces whereas on a meat-containing diet coprosterol and dihydrocholesterol are the principal fecal sterols. As judged by feeding experiments, phrenosin or some other active factor was present in greatest amount in brain, and detectable in eggs and in other organs but not in muscle (horse) or in milk. One experiment indicated the activity to be due to the fatty acid, phrenosinic acid $CH_3(CH_2)_{23}CHOHCOOH$.

The possibility that addition of phrenosin to the diet might protect against atheromatosis has apparently not been intensively investigated. However, in a recent study cholesterol was fed to rabbits as a 15 per cent solution in a commercial cholesterol-free brain lipid preparation at a level of about 100 mg. of cholesterol, and presumably somewhat more than that amount of phrenosin, per rabbit per day.[72] Nothing unusual was noted in the speed of development or the severity of atheromatous lesions.

The conversion of cholesterol to coprosterol in the colon apparently proceeds via cholestenone (Fig. 1, v), and the conversion in the tissues to dihydrocholesterol may also follow this pathway since Anker and Bloch[38] found





2A 2B

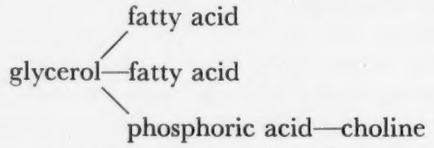
FIG. 2. A and B, Structural formulas of typical members of the neutral fat and the four compound lipid families. The hydrocarbon chains of —CH_2— groups have been represented using the same convention as for sterols to give a clearer picture of the actual molecular shape. The compound lipids have structures analogous to those of soaps, detergents and other surface active substances in that they have strong hydrophilic groups fastened to long hydrophobic carbon chains. They are all variations of the same pattern in which the water solubilizing moiety may have one, two or three charged groups or none as in the cerebrosides. This last corresponds to a non-ionic detergent such as the "Tween" group, the concentration of hydroxyl groups being responsible for the hydrophilic properties.

that feeding labeled cholestenone to rats resulted in the formation primarily of labeled dihydrocholesterol rather than of labeled cholesterol. In the laboratory cholesterol on hydrogenation gives only dihydrocholesterol while cholestenone gives coprosterol. Although the functional relationship of cholesterol and dihydrocholesterol is not known, it is of interest to note that a number of other lipids occur as mixtures with their dihydro derivatives, such as oleic and stearic acids, palmitoleic and palmitic, and also some phospholipids. The percentage of the dihydro form in tissue cholesterol is constant and very low in most species but in the mouse it is about 10 per cent.[48]

Compound Lipids. This group includes phospholipids, cerebrosides and practically all the other known lipids composed of three or more different constituents. All members contain fatty acids and either N or P compounds or both; the two chief sub-groups are the *phospholipids* and the *cerebrosides.*

I. *Phospholipids* (or phosphatides) contain phosphorus and have been customarily classified into three types, largely on the empirical basis of solubility differences:

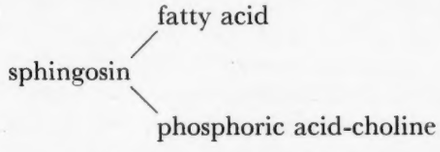
1. *Lecithins* are soluble in alcohol and ether but insoluble in acetone, and constitute a single family of compounds with the structure shown in Figure 2A, II, and represented schematically as:



A number of different fatty acids may be present in lecithin as in other phospholipids.

2. *Cephalins* are soluble in ether but insoluble in alcohol or acetone. Several families are known, all having the same structure as lecithin except that the place of choline is taken by other substances, including ethanolamine, serine or inositol. This constant part of the molecule is phosphatidic acid, and Folch[73] has suggested naming lecithin and all the cephalins as phosphatidyl derivatives. Structural formulas are given in Figure 2A, III, for the two best known.

3. *Sphingomyelins* (Fig. 2B, IV) are soluble only in hot alcohol; they contain in place of glycerol the amino alcohol sphingosin in the linkage:



They are diaminophospholipids since both sphingosin and choline are N-containing.

II. *Cerebrosides* (or glycolipids) resemble sphingomyelins in solubility properties and in being derivatives of sphingosin. Since they contain galactose in place of the phosphoryl-choline group,

they cannot be included in the phospholipids. A structural formula is given in Figure 2B, V.

The phospholipids are found in all living tissues and must be considered as essential for life. They probably exercise a variety of functions in animals, including a role in permeability of cell membranes, in muscular activity particularly of voluntary and cardiac muscle, in function of nervous tissue, in immunologic reactions, etc. Plasma phospholipids do *not* transport fatty acids from one tissue to another as previously thought. One of their functions in plasma seems to be concerned, in some way not well understood, with the maintenance of all the plasma lipids in a state of optical clarity. All cells in the body are apparently able to synthesize their own phospholipids if provided with essential fatty acids, either choline or some source of labile methyl groups, and inositol. Lecithin is the most abundant compound lipid in most tissues and the cephalins next (except in plasma), with sphingomyelins usually present in considerably smaller amount. In mammals plasma is exceptional in containing only a small amount of cephalin. Until 1944 cephalin was generally accepted as a major plasma phospholipid but recent improvement in methods of separation and analysis have shown that human plasma phospholipids consist of about 80 per cent lecithin, 3 to 8 per cent cephalins and about 15 per cent sphingomyelin.[42–44]

Cerebrosides are more widely distributed than is the common impression, being present in small amounts in many if not all tissues and in large amount in brain. Little is known about their function or metabolism, but it is of interest to note that in Gaucher's disease cerebrosides containing glucose instead of galactose accumulate markedly in liver and spleen.[40]

The ratio of cholesterol to total phospholipid in plasma has recently received much attention since it is normally about 1.6 on a molar basis and increases in hypercholesteremic states associated with atheromatosis. It is therefore of interest that this ratio invariably is lower than 1 in all other tissues, being about 0.2 in liver, 0.12 in skeletal muscle, 0.5 in smooth muscle and 0.9 in red cells. The problem of attempting to decrease the C/PL ratio in plasma might be attacked by investigation of the factors regulating the distribution of phospholipids between tissues and plasma.

FATTY ACIDS

Each of the above lipids may contain several different fatty acids and therefore must be considered as a family of closely related but not chemically identical substances. Three groups of fatty acids may be recognized:

Common Non-essential Fatty Acids. Oleic, palmitic, stearic and palmitoleic acids are all 16 or 18 carbon acids with no or only one double bond, and are the most abundant fatty acids. They can all be synthesized in all tissues of the body from other fatty acids or from carbohydrate or protein. They are found in neutral fats, phospholipids and cholesterol esters. A number of other fatty acids fall in this category but are not enumerated here since they are of less importance.

Essential Fatty Acids. Linoleic, linolenic and arachidonic acids, containing 2, 3 and 4 double bonds respectively, are essential fatty acids because they cannot be synthesized by animals, according to available evidence, and at least

one of them must be present in the diet to prevent the development of deficiency symptoms, particularly changes in the skin. They occur also in all three types of lipids but there is a highly significant difference in distribution; cholesterol esters are predominantly highly unsaturated, lecithin and cephalin fatty acids somewhat less so, and neutral fat still less. In fact the fatty acids combined with cholesterol have such high iodine values that they must be largely in the essential category, suggesting that one function of cholesterol in bile and intestinal secretions is to assist in the absorption and transport of essential fatty acids. Supporting evidence is that cholesterol is known to be esterified during absorption and that total plasma cholesterol has been reported to rise following a meal high in olive oil but free of cholesterol;[74] this interesting question has not, however, been intensively investigated.

Specialized Fatty Acids. The cerebrosides and sphingomyelins contain fatty acids, most of which are not found in any other lipids, such as lignoceric, phrenosinic, nervonic and cerebronic. They all have more than 20 carbons and some have α-hydroxyl groups; their metabolism is obscure.

Changes in the nature of the fatty acids in neutral fats and in the intestinal and liver lecithins and cephalins can be effected by dietary means but fatty acids in other lipids are relatively independent of the nature of the diet, particularly cerebrosides and sphingomyelins.

NATURE OF LIPIDS IN NORMAL AND ATHEROSCLEROTIC AORTAS

Since Aschoff's first observation of the high cholesterol content of atherosclerotic aortas, several studies of the lipids in normal and diseased aortas have been made. Hirsch and Weinhouse[24] and MacArthur[45] recently reviewed the field and give data on the composition of the intima and media, early plaques, ulcerated plaques, etc. It has been recognized that the composition of the lipids in early plaques is so similar to that of plasma as to suggest that the atheromatous lipids are derived from the blood, not formed in the tissues. A more definitive answer to this question can now be obtained by the use of labeled cholesterol and results of interest will no doubt be forthcoming.

The most interesting chemical differences between normal and atherosclerotic aortas are (1) the higher content of cholesterol esters in the latter and (2) the higher content of saturated sterols as compared to total cholesterol. It has been recognized for some time that the "clefts" observed in human arteries are actually cholesterol ester crystals and MacArthur's isolation from arteriosclerotic aortas of pure cholesterol palmitate is of interest because esters of saturated fatty acids would not be expected to be present in plasma or tissues except in very small amount. As mentioned above, cholesterol esters have very high iodine values, 158 according to Bloor,[46] corresponding to an average of about 1.7 double bonds per fatty acid. The accumulation of palmitate might be due to the fact that it is less soluble than unsaturated fatty acid esters and is therefore not as rapidly reabsorbed from the sub-intimal lipid deposits, or it might be an abnormal plasma constituent.

Very little is known about the nature of the fatty acids present in cholesterol esters in disease and this would appear to be a promising field for investigation now that a method for separating neutral fat from cholesterol esters by means of a lipase specific for the former is available.[47]

Both Schoenheimer[48] and MacArthur[45] found the total cholesterol fraction from arteriosclerotic aortas to contain about 10 per cent of dihydrocholesterol, or several times as much as any other tissue. There are two other conditions in which high concentrations of the saturated sterol in humans have been reported. Boehm in 1911 reported his studies on a closed, sterile loop of intestine formed as the result of surgery fourteen years previously; the waxy contents were fractionated and pure dihydrocholesterol free from cholesterol was found in considerable amount.[49] On the basis of this observation Schoenheimer made sterile fistulas of both large and small intestine in dogs[48] and established the fact that dihydrocholesterol is a metabolic product which is excreted in intestinal secretions and not reabsorbed.

An interesting case of idiopathic hypercholesterolemia was studied by Schoenheimer[50] who found that the plasma cholesterol contained 10 per cent of the dihydro derivative. In this case the cause of the hypercholesterolemia was shown to be an inborn inability to excrete cholesterol in the feces. On a mixed diet the plasma level was 850 mg. per cent, of which only 50 mg. per cent was free, and the fecal excretion of total sterols was about 113 mg. per day or only 0.1 of normal. When put on an all-

vegetable diet the patient's fecal sterol excretion did not change at all but the plasma level fell gradually to around 300 to 350 mg. per cent. No cholesterol or coprosterol could be identified in the fecal sterol fraction on either diet, and it was concluded that it consisted entirely of mixtures of vegetable sterols.

It appears then that dihydrocholesterol is an end product of cholesterol metabolism which is normally kept from accumulating by excretion through the intestinal wall. It does accumulate in arteriosclerotic lesions, perhaps because the deposits are relatively stagnant and excretion is therefore hindered.

Other steroids have been looked for in atheromatous aortas, particularly in the non-saponifiable fraction. Cholestenone was identified spectroscopically[51] and a number of other oxidation products of cholesterol isolated in trace amounts;[52] but, as the authors point out, most of them may have been formed from cholesterol during the isolation procedures. Cholesterol is rather easily oxidized in colloidal solution even at room temperature by an air current; at 85°C. after two hours only 20 per cent is left unchanged and 7-ketocholesterol appears to be the primary product.[53] The non-saponifiable fraction* of atherosclerotic aortas contains about 76 per cent of total sterols[48] as judged by digitonin precipitation and there are probably unidentified compounds in the residual 24 per cent.

A newly recognized end product of cholesterol metabolism in rats is an acid which Cook[54,55] isolated from the feces of cholesterol-fed animals. Although its structure is not yet known, it appears to contain the original 27 carbon atoms of cholesterol but has a carboxyl group. It is formed in the tissues rather than in the intestine since it appears only if the cholesterol is absorbed. Evidence for the occurrence in feces of an acid derived from cholesterol even on a low cholesterol dosage has been obtained by feeding C^{14}-labeled cholesterol.[56] Preliminary experiments indicate that this may represent an important route of metabolism and excretion of cholesterol.

* The non-saponifiable fraction consists of all the substances in tissues which are not converted into soaps by alkaline hydrolysis and are soluble in ether but insoluble in water. It consists mainly of cholesterol and small amounts of other sterols but also contains fat-soluble vitamins, hydrocarbons, higher alcohols and unidentified substances. The non-saponifiable fraction from human tissues has the following sterol content: blood, 98.6 per cent; liver, 70 to 80 per cent; kidney, 88 per cent; brain, 81 per cent; adrenals, 46 per cent; and atherosclerotic aorta, 76 per cent.[48]

ORIGIN OF PLASMA LIPIDS

A fundamental question in arteriosclerosis is the site of origin of plasma cholesterol and other lipids, whether one is considering the disease from the standpoint of simple hypercholesterolemia, of cholesterol-phospholipid ratio or of abnormal lipoproteins. In spite of a general belief that the liver is the source of plasma lipids, Mann and Bollman's classical studies on hepatectomized dogs failed to show any significant changes in plasma cholesterol level during the entire period of survival of about twenty to thirty hours.[57] This is not surprising since we now know that the average life of a plasma cholesterol molecule is about twelve to fourteen days, so that only a small decrease in level would be expected in one day. There is much indirect evidence to indicate that the liver does contribute to plasma lipids but it is not clear whether other tissues also contribute, and what tissues are involved in the regulation of the plasma cholesterol concentration. Recent *in vitro* studies have demonstrated that most tissues of the body can synthesize fatty acids, phospholipids and cholesterol.

In vitro measurements of lipid synthesis can be made only with the aid of isotopic tracers since there is never a significant increase in the total amount of lipid in the tissue during the experiment, and there may be a net decrease. If tissue slices are incubated with a suitable precursor such as C^{14}-labeled acetate or P^{32}-labeled phosphate for several hours and the lipids subsequently isolated prove to contain isotope that cannot be removed by extensive purification, it may be concluded that synthesis of lipid molecules must have taken place. By this type of technic phospholipid synthesis has been demonstrated in surviving slices of liver, kidney, small intestine, muscle, brain and nerve.[25]

Cholesterol synthesis was first detected in rat liver slices by Bloch, Borek and Rittenberg[58] and later in beef adrenal cortical tissue, rat skin, intestine, kidney, testes and in the brain of day-old rats by Srere, Chaikoff and associates.[59] We have confirmed these results with dog and rabbit tissues, and human skin, and also have found cholesterol synthesis to occur *in vitro* in rat diaphragm.[67] In fact, the only tissues that have failed to give any evidence of ability to

synthesize cholesterol are brain slices of adult animals and plasma[80] and dog aorta.[67] No synthesis has been reported in cell-free homogenates, although fatty acid oxidation can be effected by isolated mitochondria.

In vitro synthesis in a given tissue merely establishes the existence of a series of synthesizing enzymes and the capacity for synthesis but does not prove that the tissue makes all its own cholesterol in the intact animal and, of course, tells us nothing about whether it contributes cholesterol to plasma or not.

Results in general accord with the *in vitro* studies have been obtained in living animals. Extrahepatic synthesis of cholesterol was demonstrated by Chaikoff and associates in eviscerated rats[59] and by Gould and associates in hepatectomized dogs.[60] Popjak's experiments in normal pregnant rabbits are also strong evidence for this thesis.[61]

Chaikoff and collaborators have shown convincingly that virtually all the plasma phospholipids are derived from liver by administering P^{32}-labeled phosphate[62] and C^{14}-labeled palmitic acid[63] to hepatectomized dogs and comparing the isotopic content of plasma phospholipid with that of control dogs. Only traces of isotope were recovered from plasma of hepatectomized dogs although other tissues, such as kidney, intestine, etc., were able to synthesize phospholipids at the same rate as the corresponding tissues of control dogs.

Very recently the application of a similar isotopic tracer method has shown that in the dog the liver is the only significant source of plasma cholesterol.[60] Sham-operated control and hepatectomized dogs were given standard amounts of C^{14}-labeled acetate and the amounts of isotope that appeared in plasma cholesterol during the next four hours were taken as a measure of the amount of newly synthesized cholesterol in plasma. The hepatectomized dogs gave no significant amount of C^{14} in plasma while sham-operated controls gave very high specific activities. That this difference was not due to shock or other secondary effect of the surgical procedure was evidenced by an essentially normal rate of oxidation of acetate to $C^{14}O_2$ and by the synthesis of cholesterol and fatty acids in extrahepatic tissues.

INTERCHANGE OF LIVER AND PLASMA LIPIDS

So many major plasma constituents are now known to originate entirely in liver, including glucose, albumin, fibrinogen, phospholipids and cholesterol, and probably also the α- and β-globulins, that the two tissues may properly be considered as different compartments of a single system. Only a few plasma proteins, such as the γ-globulins, are apparently formed primarily outside the liver. The close relationship between liver and plasma in regard to lipids is strikingly brought out by the studies of Hevesy[64] and Zilversmit, Chaikoff et al.[65] on the movement of labeled plasma phospholipid molecules into the liver. If plasma containing P^{32} labeled phospholipids prepared biosynthetically in one animal is injected into a second, it will be found that the labeled phospholipid molecules leave the plasma rapidly and a large fraction may be recovered from the liver within a few hours. Since there is no detectable change in the total content of phospholipids in either plasma or liver during this period, this clearly is the result of a rapid exchange or equipartition, as Hevesy has called it, of phospholipid molecules between plasma and liver. The half-life of labeled plasma phospholipid in the plasma in these circumstances was found to be about one and one-half hours in the rabbit[64] and five to eight hours in the dog.[65] Of the total amount of labeled phospholipid introduced, 50 to 55 per cent was in plasma, 11 to 16 per cent in liver and the rest distributed in small amounts in intestine, kidney, muscle, etc., at the end of five hours. Altogether 75 per cent of the original amount was accounted for. That this rapid loss of isotopic phospholipid from plasma was due chiefly to movement into liver was shown very ingeniously by Entenman, Chaikoff et al.[65] who removed the liver or rendered it non-functional during the equilibration. An abrupt sharp decrease in this rate of disappearance to about 10 per cent of the former value was observed. The half-life of labeled plasma phospholipids in the hepatectomized dog was found to be so long (about two and one-half days) that one may conclude that plasma phospholipids enter tissue cells much more slowly than liver.

The equilibrium between the two is reached from the other side merely by administering a phospholipide precursor such as P^{32}. At the end of six hours Chaikoff[25] found the liver to contain twice the concentration of P^{32} in phospholipids as plasma but by eighteen hours they were equal and remained equal thereafter. Since the rate of equilibration is considerably faster than the rate of utilization, either in the liver or outside, they

remain in equilibrium. Equilibration between liver and plasma is a highly selective process since lecithins and sphingomyelins account for 95 per cent of plasma phospholipids and liver cephalin is therefore hardly involved.

C^{14} content. Plasma esterified cholesterol, however, had very little isotope at one hour, indicating that free cholesterol is the primary product of synthesis in the liver and is released into the plasma prior to esterification.

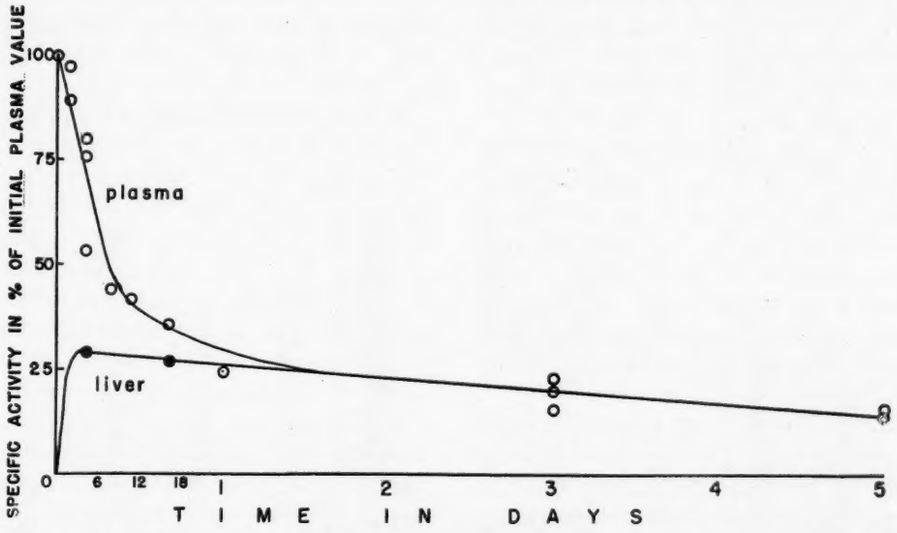


FIG. 3. Equilibration of isotopic cholesterol between plasma and liver. Data from four dogs given transfusions of blood containing C^{14}-labeled cholesterol in lipoprotein form. Blood samples were taken at frequent intervals and the dogs sacrificed at four hours, seventeen hours, five days and fourteen days. All values shown are the specific activities of the cholesterol isolated from plasma and liver, expressed in per cent of the value for the plasma sample taken ten to fifteen minutes after the completion of the transfusion.

The rates of regeneration of individual liver phospholipids have been measured in the intact dog by liver biopsies taken at different time intervals.[66] Lecithin was found to have the fastest regeneration rate (in per cent of the amount present) and sphingomyelin the slowest, but all three were of the same order of magnitude.

Similar studies on plasma cholesterol have recently been carried out, the rate of movement of isotopic cholesterol from liver to plasma being measured after administering C^{14} labeled acetate and the rate of movement from plasma to liver after injecting blood containing isotopic cholesterol in lipoprotein form.[60] The results are similar to those for phospholipids in most respects but show even faster rates of equilibration.

When a single dose of labeled acetate was administered to a dog by stomach tube, labeled free cholesterol was found in plasma in a few minutes and in fact the specific activity reached a maximum value within an hour. The cholesterol level itself did not change perceptibly. In a dog sacrificed at one hour, and in several sacrificed at four hours, the free cholesterol of plasma and of liver was found to have the same

Equipartition of newly synthesized cholesterol molecules between free plasma cholesterol and both red cell and plasma ester cholesterol took place within a few hours, and then remained in equilibrium indefinitely, indicating that the rate of utilization of cholesterol by tissues is slower than the rates of attainment of equilibrium of these various forms of liver and blood cholesterol.

The regeneration rate of cholesterol in the liver-blood system was determined from the decrease of specific activity during the week or two following this initial period of interchange and was found to be about eight days for 50 per cent regeneration, as described below.

The equilibrium between blood and liver cholesterol may be reached from the other side, starting with isotopic cholesterol in blood. A donor dog was given C^{14}-labeled acetate (300–500 microcuries of C^{14}) and exsanguinated sixteen hours after the last dose to ensure the absence of isotopic acetate and the equal distribution of isotope in all forms of blood cholesterol. Some of this blood was then transfused into a recipient dog as replacement for an equal

quantity of the recipients's own blood, thus keeping the total amount of cholesterol in the animal essentially constant. The recipient dogs were sacrificed, exsanguinated and perfused with saline at various time intervals and the distribution of labeled cholesterol in liver and other tissues determined. As in the case of phospholipids, the labeled cholesterol left plasma rapidly during the first few hours, primarily to enter liver cells, as may be seen in Figure 3. The equilibration of total plasma cholesterol with that of liver was found to be 50 per cent complete in about eight hours and equilibration of free plasma cholesterol was even faster. In other words the specific activity of plasma free cholesterol fell off more rapidly than the red cell free or plasma esterified forms, indicating again that it is metabolically the most active component of the three.

After equilibration of blood cholesterol with liver, the rate at which the specific activity decreased became very slow, eventually reaching about the same value as found for the dogs given labeled acetate or about eight days for a 50 per cent decrease.

In the transfusion experiments the cholesterol of all tissues gradually reached the same isotope concentration as plasma, suggesting that a slowly established equilibrium may exist between plasma and extrahepatic tissue cholesterol. However, in studies on hepatectomized dogs, extrahepatic tissue cholesterol was found unable to enter the blood in appreciable quantity during the four- to eight-hour periods studied, suggesting that movement of cholesterol from plasma to tissues is an irreversible process, and the bulk of the evidence available at present favors this alternative; more extensive work will be necessary to establish whether or not normal tissues (including arteries) are constantly exchanging cholesterol molecules with plasma at a slow rate, but it can be definitely stated from the above experimental findings and also from other experiments in rats fed C^{14}-labeled cholesterol that plasma cholesterol does enter tissue cells.

CHOLESTEROL REGENERATION RATE

London and Rittenberg recently reported that the rate of turnover by endogenous synthesis (here defined as regeneration rate*) of serum cholesterol in a normal human as determined by the deuterium technic was at the rate of 50 per cent in eight days,[68] a value very close to that found in dogs given labeled acetate.[69] This corresponds to a regeneration time of twelve days or a rate of regeneration of serum cholesterol in this particular subject of about 546 mg. of cholesterol per day ($\frac{1}{12}$ of total mass of serum cholesterol).

If a rapid interchange of cholesterol molecules between plasma, liver and red cells takes place in man as it does in dogs, the actual rate of synthesis would be one-twelfth of the combined cholesterol of all three tissues, or about 1.2 gm. per day. The amount of cholesterol synthesis in extrahepatic tissues is difficult to estimate but will certainly increase the above figure appreciably. A conservative estimate would be about 0.5 gm. per day,* making a total endogenous rate of synthesis in the neighborhood of 1.5 to 2 gm. per day, or considerably more than is present in the average diet. If man has a homeostatic mechanism for regulating plasma cholesterol similar to that of the dog, it would be reasonable to expect that amounts of dietary cholesterol present in ordinary American diets could be completely compensated for by a throttling of hepatic synthesis. While this conclusion may help to explain the absence of a marked rise in plasma level following increased dietary consumption of cholesterol, it should not be taken as predicting the effect of restriction of cholesterol intake on the course of atheromatosis since many other factors are also involved.

INFLUENCE OF DIETARY CHOLESTEROL ON ENDOGENOUS CHOLESTEROL SYNTHESIS

When large amounts of cholesterol are fed to experimental animals, the homeostatic mechanisms of the body which attempt to maintain a constant concentration in plasma and the body as a whole have three possible methods of

* Regeneration time is the time required for synthesis of an amount of the component equal to the total present. It is given by 1.44 × the 50 per cent regeneration time as determined experimentally and is the same as the average life of a molecule. The term "regeneration" is used to denote replacement by newly synthesized molecules since "turnover" implies only replacement without regard to source and can be used appropriately to denote, for example, replacement of tissue cholesterol molecules by labeled dietary cholesterol.

* This is calculated by assuming that the total cholesterol content of other tissues (excluding central nervous system) is about 50 gm., that its regeneration rate is about one-fifth that of liver, as in the case of the rat (Pihl, A., Block, K. and Anker, H. S. *J. Biol. Chem.*, 183: 441, 1950), and that about half of the tissue cholesterol is derived from plasma and half from intracellular synthesis.

reacting: (1) absorption may be decreased, (2) endogenous synthesis may be inhibited or (3) the various methods of disposal of cholesterol may be accelerated. There is no evidence that the body fails to absorb cholesterol when given in moderate amount, provided that sufficient amounts of other lipids are present in the diet, as they invariably are in natural foods. In balance experiments a discrepancy between the total cholesterol initially (in animal + diet) and the total amount terminally (in animal + excreta) has been taken as proof of increased cholesterol destruction. The same result would be produced by a decrease in endogenous synthesis so that it is not possible to distinguish between these two alternatives by balance experiments alone. The isolation from rat feces of considerable amounts of an acidic product apparently derived from the large quantities of dietary cholesterol already referred to[55] indicates an increase in the rate of disposal. However, evidence that a decrease in synthesis occurs in dogs, particularly in liver, has been obtained by measuring the incorporation of labeled acetate in cholesterol, both in intact animals[86] and in tissue slices.[87] In the former method pairs of littermates were fed identical diets except that one was given 1 gm. of cholesterol per day for seven to ten days. Twenty hours after the last feeding standard doses of isotopic acetate were given to each and four hours later they were sacrificed and cholesterol isolated from a number of tissues. The striking differences in amount of newly synthesized cholesterol in the liver (and in plasma) between cholesterol-fed and control animals are shown in Figure 4.

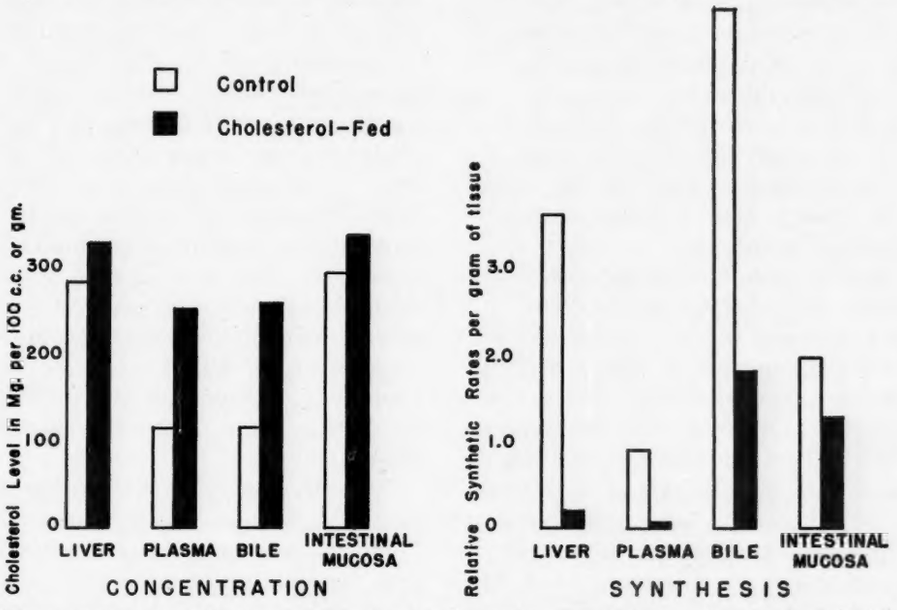


FIG. 4. Influence of dietary cholesterol on cholesterol concentrations and rates of synthesis. The left side shows the effect of feeding 7 gm. of cholesterol to a 4.5 pound dog during a period of eight days as compared with his littermate on a cholesterol-free diet, otherwise identical. The right side shows the relative amounts of isotopic cholesterol formed per gram of tissue following standard isotopic acetate administration.

The results are expressed in units proportional to the amount of isotopic cholesterol present per gram of tissue and are thus independent of the total amount of cholesterol present. In another experiment the effect of a single 4 gm. dose of cholesterol was approximately as great. As may be seen from Figure 4, the effect was most pronounced in the liver and was of doubtful significance in many of the other tissues. The cholesterol of plasma and bile, being wholly derived from liver, were affected to approximately the same extent by diet as the liver. Fatty acid synthesis was not affected significantly by cholesterol feeding in liver or in any tissue studied, indicating that the inhibitory effect on hepatic synthesis was not due to non-specific liver damage.

Similar results were obtained *in vitro* by incubation of tissue slices from control and cholesterol-fed animals with isotopic acetate. Synthesis was 50 to 100 times faster in control liver slices than in experimental, but rates in

other tissues studied showed relatively small differences. The plasma cholesterol level may be ruled out as a possible controlling factor in the rate of synthesis in these *in vitro* experiments and liver concentrations did not vary significantly in most cases; hormonal effects, however, have often been shown to persist in surviving tissue slices even after thorough washing to remove any diffusible hormone present.

It thus appears that in the dog hepatic cholesterol synthesis can be reduced from its normal rate to almost zero and that this represents a very considerable decrease in endogenous cholesterol production.

Rabbits showed qualitatively the same type of effect in liver slice studies; however, the rate of cholesterol synthesis in the control animals was far slower than in dogs so that a decrease was of much less quantitative significance. Cholesterol metabolism appeared to be much slower in rabbits than in dogs, both the rate of synthesis and the equal rate of disposal, so that when cholesterol was fed, rabbits were apparently less able to compensate by a decrease in synthesis.

One interesting species difference which has been observed in several laboratories is the relatively rapid rate of cholesterol formation in the small intestine of rabbit and rat as compared with liver. Popjak and Beekmans found that even following intravenous administration of acetate to rabbits, intestinal cholesterol had a higher specific activity and therefore probably a higher rate of regeneration than liver,[61] whereas in the dog neither whole intestine nor intestinal mucosa has been found to be very active in cholesterol synthesis. A possible explanation of this observation is that rabbits, being habituated to a cholesterol-free diet, may have need for the secretion of larger quantities of cholesterol into the intestine by intestinal mucosa than do dogs. In both species intestinal synthesis was unaffected by dietary cholesterol. Rabbits with severe cholesterol-induced atheromatosis still showed some cholesterol synthesis by the intestine although all other tissues studied gave barely detectable amounts of isotope.[86]

INFLUENCE OF THE THYROID ON CHOLESTEROL METABOLISM

The thyroid gland is undoubtedly one of the regulators of plasma cholesterol level and probably of plasma phospholipid level as well; its relationship to lipid metabolism in tissues is largely unexplored. It has been reported[75] that the total body content of cholesterol is unchanged in thyroidectomized animals despite a more than twofold increase in serum level; this suggests that the thyroid is concerned with the distribution of cholesterol between plasma and tissues rather than with total body synthesis or utilization. There was even a suggestion of an inverse relation between serum and tissues since in thyroid-fed rats and rabbits serum cholesterol was lower than normal and whole body cholesterol content somewhat higher. While this latter effect may be due to changes in neutral fat content, extension of these investigations to individual organs, especially liver, would be of interest. In preliminary experiments on rates of cholesterol synthesis in thiouracil-fed dogs we have found increases in cholesterol content of both plasma and liver in thiouracil-fed dogs to about double the value in littermate controls.[69]

Recently, the possible therapeutic effectiveness of iodide in the treatment of hypercholesterolemia has received considerable attention. It is therefore pertinent to point out that results in experimental animals on the effects of iodine, iodides, thyroxin and desiccated thyroid on hypercholesterolemia and on atherosclerosis from cholesterol feeding are far from clear-cut at present; iodides in some cases have increased cholesterol levels and possibly aggravated the disease. Rosenthal fed potassium iodide to rabbits at levels of 2 to 3 mg. iodine per day in addition to cholesterol and found a dramatic increase in serum cholesterol over that on cholesterol alone, levels of 2,500 to 3,000 mg. per cent being reached in about ten weeks.[76] The degree of atheromatosis was greater in the iodide-treated animals and appeared to be proportional to the cholesterol levels, the duration of hypercholesterolemia being constant. Page and Bernhard,[77] Turner[78] and others have found a protective action of iodides or organic iodine compounds against cholesterol induced atherosclerosis but the doses given were very large. The effect of iodide administration is complex, depending on dose and also on the condition of the gland and, while often beneficial, is not entirely without hazard.

While thyroid hormones influence plasma lipid levels, they also influence the metabolic activity of tissues, including aorta, and the possibility that this latter action may change the course of the disease should not be overlooked.

Briggs et al.[79] have recently studied the oxygen consumption of rat aorta *in vitro* and found it to be about 10 per cent that of liver in the normal. Previous feeding of thyroid increased and of propyl thiouracil decreased the *in vitro* O_2 consumption. Aorta apparently contains the enzymes oxidizing the Krebs cycle compounds (succinate, citrate, pyruvate, etc.) and is capable of synthesizing fatty acids and phospholipids[80] but no evidence of cholesterol synthesis has been obtained.[67]

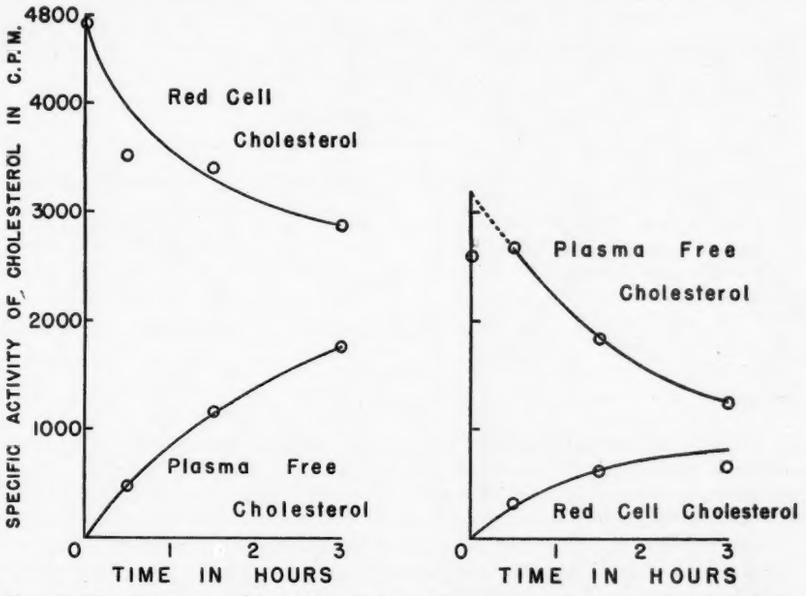


FIG. 5. Equilibration of isotopic cholesterol between plasma and red cells *in vitro*. Blood from a dog fed C^{14}-acetate was separated into red cells and plasma. The cells were incubated at 37°C. with unlabeled dog plasma as shown on the left and the plasma with unlabeled red cells. Only the specific activities of free cholesterol are shown. Equilibration was rapid, the final specific activity depending on the relative amounts of labeled and unlabeled free cholesterol in the system.

STABILITY OF PLASMA LIPOPROTEINS

Most of the recent work on plasma lipoproteins has supported the concept that they are definite chemical compounds rather than adsorption complexes since the composition of different preparations has been found to be essentially uniform[31] and the lipids are not extractable with ether under ordinary conditions unless the protein has been denatured. However, special methods for removing lipids without denaturation have been known (but not often used) since Hardy and Gardiner in 1910 reported that treatment of plasma with large amounts of alcohol or acetone at −8°C. and subsequent extraction with ether would remove all the lipids without affecting any other physiologic attributes of plasma proteins.[81] Subsequently, it has been found that the electrophoretic pattern of delipidized plasma or serum differs in having a smaller β-globulin peak. Blix[82] has reported that a colloidal suspension of cholesterol added to delipidized serum appeared to recombine with β-globulins as judged by the observation that an opalescent zone of colloidal cholesterol traveled with the β-globulin fraction on electrophoresis. This does not, of course, prove that the original lipoprotein has been reconstituted in chemically identical form but it does suggest that certain β-globulins have a special affinity for lipids and can combine with them even in the presence of other proteins. There is evidence that water is an essential component of lipoproteins since they are most readily split by procedures that remove the water, such as freezing below −25°C. or treating with alcohol or acetone.

Evidence that cholesterol is in extremely labile and rapidly exchangeable form in normal lipoproteins of plasma, liver and red cells has recently been obtained by means of the C^{14} tracer technic.[83] No solvents or other chemical treatments were used and the exchange was observed to occur at approximately the same rate *in vivo* and *in vitro*. In the latter case blood

from a dog previously given C^{14}-labeled acetate was separated into cells and plasma; the red cells containing C^{14}-labeled cholesterol were mixed with plasma from a second dog and incubated at 37°C. for several hours.

incubation of C^{14}-labeled plasma with unlabeled red cells, gave similar results. The free cholesterol of plasma exchanged rapidly with red cell cholesterol molecules until both reached the same specific activity, while the ester was

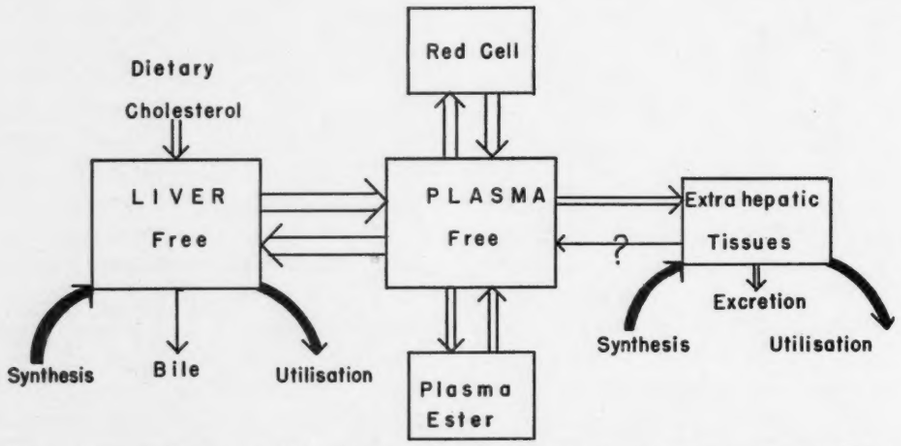


FIG. 6. Some pathways in cholesterol metabolism. Solid arrows indicate formation and destruction (or conversion into other substances) of cholesterol molecules and open arrows indicate movement or interchange. Width of arrows represents very roughly the relative rates of the various processes. Cholesterol excreted in bile or intestinal secretions becomes identical with dietary cholesterol and may be either reabsorbed, excreted or converted to coprosterol. Phospholipid metabolism is very similar except for the limited exchange between plasma and red cells.

As shown in Figure 5, the free cholesterol of plasma acquired radioactive cholesterol rapidly while the red cells lost activity, until after three or four hours both had reached about the same concentration of labeled cholesterol although no significant change in the actual cholesterol

TABLE III
PHOSPHOLIPIDS IN HUMAN BLOOD*

	Plasma	Red Cells
Total, in mg, per 100 ml	229	309
Lecithin, in per cent of total	78	29
Cephalins, in per cent of total	5	41
Sphingomyelin, in per cent of total	17	28

* Data from Hack[43]

content of either tissue took place. Plasma esterified cholesterol, on the other hand, acquired very little isotopic cholesterol; red cells were almost devoid of cholesterol esters so that direct exchange was not possible and the equilibrium between free and esterified cholesterol must have been very slow. The converse experiment,

inert. There is little possibility that the exchange involves the intact lipoprotein molecule for several reasons: (1) red cell lipids are associated with the insoluble stroma rather than the soluble proteins as in plasma; and (2) both free and esterified cholesterol are part of the β_1-lipoprotein molecule, which constitutes 75 per cent of the total plasma lipoproteins, and only the free cholesterol exchanges.

The rate of movement was about 1 to 2 per cent per minute, corresponding to the exchange of about 10 mg. of cholesterol per minute between the cells and plasma of a liter of average blood.

Similar *in vitro* experiments on phospholipids with P^{32} as a tracer were carried out in 1939 by Hahn and Hevesy who found very little interchange.[84] Since the nature of the phospholipids in plasma and red cells is now known to be quite different, as is seen from Table III, it is evident that complete equipartition of total phospholipid P could not be expected to occur and results on individual phospholipids would be of interest. Hahn and Hevesy observed that some interchange took place rapidly but that an equal distribution of isotopic tracer was never attained either *in vitro* or *in vivo*.

REGENERATION RATES OF PLASMA LIPOPROTEINS

By means of double labeling of plasma lipoproteins, the cholesterol with C^{14} and the protein with S^{35}, regeneration times for the cholesterol and protein moieties have been estimated in normal dogs;[85] the total plasma lipoprotein fraction, separated by the method of Kunkel,[32] was studied and preliminary results have given a regeneration time for the cholesterol component of twelve days and for the protein of only six to eight days.

It seems evident from all the foregoing experimental findings that the cholesterol in dog plasma lipoproteins is very labile and capable of rapid exchange, thus differing from the prosthetic group of typical conjugated proteins such as hemoglobin. The linkage between cholesterol and protein is undoubtedly of an unusual type and appears similar in certain respects to that in "choleic acids." Desoxycholic acid combines in definite proportions with a number of compounds including some that resemble cholesterol esters in having no free groups capable of forming conventional chemical linkages, such as esters, ethers and even hydrocarbons like xylene, naphthalene and the paraffins. The resulting choleic acids are soluble in water (as sodium salts) and are remarkably stable compounds. However, one substituent in a choleic acid may be readily replaced by a second, suggesting the same kind of lability of binding as described above for lipoproteins. Although proteins obviously do not contain desoxycholic acid, they may conceivably contain spatial arrangements of groups capable of forming linkages similar to those in choleic acids.

Figure 6 summarizes some inter-relations of plasma and tissue cholesterol, emphasizing the key position of the liver in the regulation of cholesterol metabolism in the body.

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Clinico-pathologic Conference

Fatal Reaction to Antirabies Vaccine

STENOGRAPHIC reports, edited by Robert J. Glaser, M.D. and David E. Smith, M.D., of weekly clinico-pathologic conferences held in the Barnes Hospital, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient, O. C. (No. 195088), a white married salesman thirty years of age, entered the Barnes Hospital on March 17, 1951, complaining of generalized weakness. The family history was irrelevant. His general health in the past had been good although at the age of seventeen he had low back pain, accompanied by weakness in both arms and both legs, which lasted for several months. When he was twenty-two he was examined for the draft in World War II and rejected because of "nerve trouble." He was placed in category 4F and was never re-examined. He had had recurrent episodes of bronchial asthma beginning at the age of thirteen; they had occurred usually in the summer and fall. In the three years prior to entry, however, he had had none and had enjoyed particularly good health.

Three weeks previous to entry the patient separated his dog from a stray dog with which it was fighting. The stray, which was believed to have been rabid, was killed but its brain was not examined. During the melee a wound on the patient's right thumb came in contact with saliva from the rabid dog. The patient consulted his physician the day after exposure and two days later received the first injection of antirabies vaccine. Subsequently he received a total of fourteen daily injections given alternately in the arms and hips. After the thirteenth injection (three days before entry) he noted some aching in both shoulders and the lower back. The following day he experienced generalized weakness and remained home from work; that night he had difficulty in swallowing. On the day before entry the patient was too weak to get out of bed and was scarcely able to move either his legs or arms. He could swallow only liquids. On the day of entry weakness was profound so that he was unable to lift his head from the pillow. The difficulty in swallowing had increased and talking became difficult. The patient also had difficulty in coughing up saliva which accumulated in his throat. Bladder and bowel control remained normal. There was no other history of recent injury, infection or known exposure to infectious disease.

Physical examination revealed the temperature to be 37.8°C., pulse 90, respirations 20 and blood pressure 118/80. The patient seemed well nourished and well developed but appeared acutely ill and apprehensive. He was able to move only the fingers and feet slightly. The skin was warm and moist and no lesions were observed. There was no generalized lymphadenopathy. The pupils reacted normally and the fundi were negative. The eardrums were intact. The patient salivated excessively; the gag reflex was present. The neck was supple. Examination of the lungs revealed them to be clear to percussion and auscultation. The heart was normal. Abdominal examination was negative and the rectal sphincter tone was good. There was no tremor, clubbing or edema of the extremities. Neurologic examination revealed a flaccid paralysis of the extremities and the neck. All reflexes were absent except for very weak ankle jerks bilaterally. No planter response could be elicited nor were abdominal reflexes demonstrable. There was slight hypalgesia and hypesthenia over the entire body from the level of the chin down. Other sensory modalities were intact as were touch and pain sensations over the face. Cranial nerve examination revealed no demonstrable abnormalities except for slight dysphagia, palatoplegia and weakness of the sternomastoid and trapezius muscles.

The laboratory data were as follows: Blood count: red cells, 5,670,000; hemoglobin, 17.0 gm.; white cells, 6,250; differential count; eosinophils 2 per cent, stab forms 3 per cent, segmented forms 72 per cent, lymphocytes 22

per cent, monocytes 1 per cent. Urinalysis: negative.

Shortly after the patient was admitted a lumbar puncture was performed. The initial pressure was 170 and the final pressure 120 mm. of water. The fluid was clear and contained no cells. The protein was 51 mg. per cent; colloidal gold and Wassermann reactions were negative.

The patient was given 100 mg. of cortisone and parenteral fluids. During the night saliva continued to accumulate in the pharynx and intermittent suction was required. Otherwise the patient appeared comfortable. At 8:30 on the morning following admission the patient began to have difficulty in breathing; it was noted that only the diaphragms were participating in respiration. Coarse scattered rales were heard throughout both lung fields. There was no response to intravenous aminophyllin and cyanosis began to develop. Tracheotomy was performed but the patient's color failed to improve, and artificial respiration as well as other emergency measures were unsuccessful. The patient died on March 18, 1951, ten minutes after tracheotomy was completed.

CLINICAL DISCUSSION

DR. HARRY L. ALEXANDER: The case under discussion today provides us with an opportunity to discuss the general problem of rabies and its prevention. This subject seems rather timely, particularly in this locale where an extensive rabies epidemic is extant. Likewise in many other areas of the country rabies becomes more of a problem in the warmer months; consequently this topic is of interest to physicians in general. We are indeed fortunate in having with us Dr. Downey Harris, a national authority on the subject, and author of the section on rabies in the Cecil-Loeb Textbook of Medicine.

In beginning this discussion I should like to ask Dr. Harford to comment on the fate of the rabies virus when it is introduced in the body.

DR. CARL G. HARFORD: It is believed that virus of rabies spreads along the peripheral nerves to the central nervous system, and from the central nervous system again via nervous pathways to the salivary glands.

DR. ALEXANDER: What is the incubation period between the occurrence of the bite and the development of the symptoms of rabies.

DR. HARFORD: The incubation period in human beings is quite variable; usually it is of the order of four to eight weeks. It may on occasion, however, be as short as two weeks.

DR. ALEXANDER: Is there any validity to the concept that the incubation period varies directly with the distance between the bite and the central nervous system?

DR. HARFORD: The incubation period is shorter for bites about the face. The reason for this observation has been disputed. Some have stated that it represents the result of a shorter pathway for the virus. Others believe that it has to do only with the amount of virus which comes in contact with nerve endings.

DR. ALEXANDER: What is the incubation period when virus is introduced into the brain of experimental animals?

DR. HARFORD: Usually the subdural inoculation of virus into the brain of dogs or rabbits produces the disease in from one to three weeks although occasionally a much longer interval may ensue before the development of symptoms.

DR. ALEXANDER: The practical problem of what to do when a person is bitten by a stray dog is an important one. Recently in this community a law has been passed which requires the immunization of all dogs. Will such a measure solve the problem, Dr. Harris?

DR. DOWNEY L. HARRIS: No, it will not. Unfortunately only dogs who are pets will be vaccinated. There are nearly as many strays in this area as there are domesticated dogs. One must couple immunization of pets with destruction of all strays.

DR. ALEXANDER: Would you comment on the effectiveness of vaccination in dogs.

DR. HARRIS: Probably 70 per cent of the dogs immunized with vaccines now available are protected. The Government currently standardizes all of the vaccines, and they are consequently more effective than formerly.

DR. ALEXANDER: How has the problem of rabies been solved in other countries?

DR. HARRIS: In England rabies outbreaks had occurred every few years. Eventually a law was passed which provided that every stray dog should be picked up and destroyed. In about six months the entire population of strays was wiped out; that measure, coupled with the law which requires quarantine of any dog brought into England for a six-month period, has eliminated the rabies problem in that country. In Australia where a six-month quarantine is enforced rabies is not seen.

DR. ALEXANDER: The elimination of stray dogs would be important then in the control of rabies.

DR. HARRIS: That is correct.

DR. ALEXANDER: If a patient is bitten by a stray dog and the dog is captured, how long should one hold the dog?

DR. HARRIS: If such a dog is observed for twenty-four to forty-eight hours, one can usually tell whether or not it is rabid. Rabid dogs do not eat and they exhibit a definite change in disposition; although it has been shown experimentally that the virus may appear in the saliva four to five days before the appearance of symptoms, it is really quite unusual for the saliva to be infectious before symptoms are recognized.

DR. ALEXANDER: I believe you have described two types of rabid dogs, one which you call the "dumb" dog and the other the "excited" type. May the "dumb" dog be infectious?

DR. HARRIS: Indeed, the saliva from "dumb" dogs is just as infectious as from those which are excited.

DR. ALEXANDER: Would you comment on the probability of demonstrating Negri bodies in rabid dogs that are killed early in the course of the disease.

DR. HARRIS: The demonstration of Negri bodies, which confirms the diagnosis of rabies, is often difficult in about 10 per cent of dogs who die of rabies. In the other 90 per cent they can usually be demonstrated without difficulty. If a dog suspected of rabies is killed early in the course of the disease, the demonstration of Negri bodies may be difficult in 20 per cent of the specimens. The longer the rabid dog lives the greater is the chance of finding inclusion bodies in the brain.

DR. ALEXANDER: If a dog suspected of rabies dies or is killed, what other diagnostic procedures may aid in confirming the diagnosis other than the examination for Negri bodies?

DR. HARRIS: The intracerebral inoculation of mice with material from the brain of a dog suspected of infection is also indicated. Mice so inoculated will usually die in six to eight days.

DR. ALEXANDER: Is it safe to withhold antirabies therapy for that long a period?

DR. HARRIS: That depends to some extent on where the patient was bitten. Severe bites on the hands or face necessitate prompt treatment. Our practice in the municipal clinic is to give the first injection and then wait until it is determined whether or not the dog is rabid; if it is, treatment is resumed.

DR. ALEXANDER: How does one proceed if a dog suspected of rabies disappears? What should be done in regard to protecting the patient in that case?

DR. HARRIS: In general it must be considered that such a dog was rabid and the patient must be treated. This is particularly true under circumstances such as exist at present in this community. It is important, however, to inquire into the circumstances of the bite. Very often one can be quite sure that a dog is not rabid if he knows these facts. It is well to remember that in a certain number of instances dogs may bite under wholly justifiable circumstances.

DR. ALEXANDER: It seems likely that many patients treated have not been bitten by dogs which are actually rabid.

DR. HARRIS: That assumption is correct. In Illinois where rabies vaccine is furnished free by the state, it is estimated that only one-tenth of the patients who are treated with antirabies vaccine have been bitten by *rabid* animals.

DR. SAMUEL C. BUKANTZ: You stated earlier, Dr. Harris, that when patients are bitten on the face or hands, and the dog has been captured you give a single injection of vaccine and then observe the dog for several days.

DR. HARRIS: That is correct. We usually wait for twenty-four to forty-eight hours during which period the dog will certainly exhibit symptoms if he is rabid. If so the treatment is resumed.

DR. BUKANTZ: One wonders whether or not the hypersensitive complications of immunization would be more likely to occur in individuals in whom an interval elapses between the first injection and the subsequent one.

DR. HARRIS: We have never observed any cases in which that seemed true.

DR. ROBERT J. GLASER: If a patient were bitten on some area of the body other than the hands or face and the dog were available for observation, would you withhold therapy for two to three days in the hope that it would not be necessary?

DR. HARRIS: That is our procedure in the municipal clinics. Of the 15,000 people who have come to our clinic since 1932 we have advised only about one-third to take treatment. To date we have never observed a case of rabies in either the treated or untreated groups.

STUDENT: What is the proper handling of a pet bitten by a dog thought to be rabid?

DR. HARRIS: If the stray dog is captured, the pet dog should be confined until it is clear that the stray is or is not rabid. If the stray is rabid, the pet should be destroyed.

DR. ALEXANDER: Dr. Levy, would you comment on the new experiments with hyperimmune globulin?

DR. IRWIN LEVY: In an attempt to improve prophylactic treatment a combination of rabies vaccine and hyperimmune antirabies serum has been used recently on an experimental basis. Recent studies have indicated that hyperimmune serum, especially when combined with vaccine, is to be preferred to the use of vaccine alone. This form of treatment is especially valuable in those individuals bitten deeply around the head and neck; in this group the incubation period may be insufficient for the development of active immunity. A field trial at Teheran is being carried on at the present time.

DR. ROBERT A. MOORE: I would like to ask Dr. Harris if there is truth to the oft-quoted statement that rabid dogs usually travel in a straight line. It is said that the simple expedient of changing one's own course, so as not to disturb such a dog, greatly reduces the chance of being bitten.

DR. HARRIS: That is entirely correct. Rabid dogs apparently suffer from delusions of persecution. At least human beings with rabies do and it is probable that the dog may also. It is wise, therefore, if one suspects that a dog is rabid, to seek other quarters.

DR. CARL V. MOORE: I should like to ask Dr. Harris how many cases of rabies in humans have been seen in the St. Louis area in the past five years.

DR. HARRIS: None.

DR. ALEXANDER: How many occur in the country per year?

DR. HARRIS: Approximately forty-eight.

DR. ALEXANDER: What is the incidence of hypersensitive reactions to therapy in man?

DR. HARRIS: Of all the patients given preventive therapy approximately 1 in 3,000 will develop some evidence of nervous system abnormality. The involvement may be very limited or widespread and indeed occasionally it may be fatal.

DR. C. V. MOORE: How many patients who have been bitten by stray dogs and were advised to take treatment refused to accept the advice? What would be the incidence of rabies in humans bitten but not treated?

DR. HARRIS: I have never known a patient who was advised to take therapy who did not do so. In the pre-Pasteur days approximately 10 per cent of people bitten by known rabid dogs died of the disease. Of those bitten on the face and head the figure was about 80 per cent, of those bitten on the hand 20 per cent, on the lower extremities 5 per cent and of those bitten through clothing the fatality rate was 0.

DR. ALEXANDER: Once the disease has developed in humans is there any therapy which may save the patient?

DR. HARRIS: No. The only patients who recover from rabies are those in whom an incorrect diagnosis has been made. The death rate in clinical rabies is 100 per cent. Of course it must be remembered that there is no way to determine whether or not patients may have recovered from rabies without having reached the symptomatic stage. The mortality figures are for patients with clinical manifestations.

DR. JOHN H. WALTERS: The patient under discussion today had scratched his hand several days previous to the time that the site was contaminated by contact with saliva from the stray dog. It was believed by his physician that this contact necessitated immunization. How likely would it have been for this patient to develop rabies had he not received the Pasteur treatment?

DR. HARRIS: If the patient had suffered the scratch several days prior to contact with the rabid dog's saliva, there was no chance of his developing rabies.

DR. ALEXANDER: Would you enlarge on that remark?

DR. HARRIS: In the city of St. Louis no contact cases such as this one are treated. If the wound is older than twenty-four hours at the time it is in contact with saliva from a rabid dog, there seems to be no chance of contracting rabies.

DR. HARFORD: I should like to ask Dr. Harris another practical question. If a physician sees a patient shortly after he is bitten, of what value is cautery or methods of washing the wound?

DR. HARRIS: The method of choice in handling a recently inflicted dog bite is thorough washing with a strong solution of tincture of green soap. A careful study in the state of Illinois revealed that experimentally inoculated dogs whose wounds were washed in that manner

had a lower percentage of takes than did dogs whose wounds were washed with various antiseptics or even strong acids.

DR. ALEXANDER: This patient developed signs of encephalitis on the thirteenth day following immunization. I have asked Dr. Jones, who has had considerable experience with such reactions, to discuss this aspect of the problem.

DR. ANDREW B. JONES: We have encountered twelve patients with neurologic disease arising on the basis of antirabies therapy in the past twenty-five years; this rather large number suggests that reactions occur with greater frequency than is usually stated in textbooks. The time of onset of the symptoms of post antirabies vaccine therapy paralysis is variable, and in part is predicated upon the type of vaccine employed; i.e., whether the material is prepared from 7 day cord, 14 day cord or 21 day cord. Commonly the symptoms appear toward the end of the course of treatment. On the other hand the onset of the illness may be delayed for weeks. Several clinical types may be observed:

1. *Acute Ascending Paralysis of the Landry Type.* In this form sudden onset with fever, headache, vomiting, excruciating dorsolumbar root pain, insomnia and restlessness are common. The next day the patient's legs are weak and there is difficulty in urinating and defecating. The facial expression is changed. Within the next few hours the pains in the back lessen but the patient becomes completely paralyzed, and there is retention of urine, a relaxed anal sphincter and paralytic ileus involving the lower bowel. Pain develops in the thorax, neck and arms. Usually by the next day the muscles of the neck and those innervated by the bulbar nerves are involved and the patient cannot talk or swallow; he becomes anxious, apprehensive and has a feeling of impending death. In other instances the patient may become stuporous. Dyspnea and tachycardia are common. In those who survive pain decreases and recovery takes place with symptoms clearing in inverse sequence to the order of their appearance. Weakness and fatigue especially of the legs may persist for a long time, but otherwise the patient regains normal function in about three weeks. The recovery in this group seems to be more rapid than in the dorsolumbar myelitic type.

2. *Dorsolumbar Myelitic Type.* Here the onset is less sudden and there is little elevation of the temperature. The patient may complain of excruciating back pain, either lancinating or of the girdle type, followed in a few hours or days by weakness of the legs, loss of sphincter control and partial or complete sensory loss up to the level of the pain in the back. In other cases of this type the first symptom may be numbness and weakness of the legs or loss of sphincter control, followed by pain in the back with concomitant anesthesia. Where there is complete flaccid paralysis of the lower extremities the cutaneous and deep reflexes are abolished. More often Babinski's sign is present. In the milder cases presenting weakness of the extremities the deep reflexes are diminished, and usually there are pathologic toe signs.

Paralysis of this type may be ascending, stopping at any level of the cord, but there are no bulbar symptoms. Trophic disturbances are common over the sacrum, heels and buttocks. Urinary infections are very common. In favorable cases after several days of the illness recovery begins.

3. *Neuritic Type.* The picture in the main is one of involvement of the peripheral nerves. More often this form of the disease begins after the end of the treatment, sometimes several weeks thereafter. The paralysis may be limited to one or both sides of the face with or without involvement of the extrinsic muscles of the eyes, disturbance in swallowing, sciatic pain and retention of urine. There may be isolated peripheral nerve palsies in any part of the body. Each case is different and no general description is possible.

4. *Meningo-encephalomyelitic Type.* This form is characterized by diffuse involvement of the central nervous system; it has an acute onset with elevated temperature, cephalalgia, rhachialgia, paralysis of the extremities and of the face, stiff neck, Kernig's sign, altered reflexes and changes in the cerebrospinal fluid. The prognosis is grave.

The treatment of all these reactions is symptomatic. The course of the illness cannot be altered by any drugs as far as is known.

DR. ALEXANDER: What is your concept, Dr. Jones, of the etiology of this type of reaction?

DR. JONES: Presumably the reaction is based on hypersensitiveness of the patient to the material injected.

DR. ALEXANDER: Thank you, Dr. Jones. Would you continue the discussion, Dr. Bukantz?

DR. BUKANTZ: Clinically the more frequent occurrence of the demyelinating encephalomyelitides after previous courses of immuniza-

tion and the fact that there are occasionally prodromata consisting of localized phenomena of hypersensitiveness preceding the development of the central nervous system lesion would indicate that there may be a hypersensitive mechanism at work. The most interesting information comes from the experimental evidence that was accumulated by Isabel Morgan and by Kabat, Wolff and co-workers with monkeys, and more recently by the group at Lederle laboratories working with guinea pigs. These studies have clearly demonstrated that the injection of either homologous or heterologous brain antigens, suspended in Freund's adjuvants, will be followed by the development of a symptom complex in monkeys and in guinea pigs that simulates closely what one sees occasionally in human beings injected with antirabies vaccine. Furthermore, the histologic lesions in these animals closely resemble those seen in the human cases; the lesions tend to be confined to the perivascular area within the central nervous system, suggesting that the lesions result from an antigen-antibody mechanism operating in the nervous tissue. Of interest, is the fact that Kabat[1] and also Moyer and his co-workers[2] have shown that these lesions can be suppressed in experimental animals by pretreatment with either ACTH or cortisone. These observations raise the question of whether the development of the serious complications of antirabies therapy may be prevented by initiating cortisone or ACTH treatment prior to the introduction of the vaccine. I discussed this with Dr. Kabat,[3] chiefly to determine whether or not one would expect at the same time to suppress the immune response to the virus, thus defeating the purpose of vaccination, or whether one might accomplish a differential suppression of hypersensitivity. There is no information on this point; Kabat's opinion was that there would be equal suppression of the immune response to the viral antigens and to the brain substance antigens which are present within the vaccine.

DR. ALEXANDER: Thank you Dr. Bukantz. Are there any other comments in regard to antirabies therapy?

DR. GUSTAVE J. DAMMIN: One of the more effective vaccines used in dogs is the chick embryo adapted living virus vaccine prepared with the Flurey strain. A single intramuscular injection protects a high percentage of dogs so treated. Since the incidence of hypersensitive reactions seems to be related to the amount of vaccine and the number of injections, it would appear logical to produce patent therapeutic as well as prophylactic vaccines in smaller bulk than the presently used cord vaccines.

DR. GLASER: Recently Koprowski and his co-workers at the Lederle Laboratories have described an anti-rabies rabbit serum which in experimental animals seems quite effective.[4] Its effectiveness depended on the use of the material within twenty-four hours of exposure. As yet adequate clinical trials have not been reported but this new material may offer another effective means of preventing rabies without the danger of causing encephalomyelitis. Whether hypersensitive reactions to rabbit serum would be of equal gravity remains to be seen.

DR. ALEXANDER: Are there any further comments?

DR. HARFORD: I believe the question as to whether this patient died of paralytic rabies may be asked, although I agree that the patient probably had encephalomyelitis as a result of therapy.

DR. ALEXANDER: Do you think that is a likely possibility, Dr. Harris?

DR. HARRIS: No, I think it would be quite unlikely.

DR. ALEXANDER: Do dogs ever exhibit symptoms of rabies as a result of other infections?

DR. HARRIS: That is a distinct possibility in some instances. I have recently isolated a virus from a dog who had atypical symptoms of rabies. In experimental animals this virus produces inclusion bodies which are identical in appearance to Negri bodies but protection is not afforded by antirabies vaccination. Furthermore the cross-immunization reactions are negative both ways.

DR. HARFORD: Isn't it true, however, that occasionally paralytic rabies does result from dog bites?

DR. HARRIS: Yes, that is true.

DR. JONES: An important aid in the differential diagnosis of rabies and post-rabies vaccine

[1] KABAT, E. A., WOLF, A. and BEZER, A. E. Effect of cortisone on experimental acute disseminated encephalomyelitis. *Federation Proc.*, 10: 412, 1951.

[2] MOYER, A. W., JERVIS, G. A., BLACK, J., KOPROWSKI, H. and COX, H. R. Action of ACTH in experimental allergic encephalomyelitis of the guinea pig. *Proc. Soc. Exper. Biol. & Med.* 75: 387, 1950.

[3] KABAT E. A. Personal communication.

[4] KOPROWSKI, H., VAN DER SCHEER, J. and BLACK, J. Use of hyperimmune antirabies serum concentrates in experimental rabies. *Am. J. Med.*, 8: 412, 1950.

encephalitis is the period between exposure and development of symptoms. This patient developed his first symptoms within fifteen days of his exposure. That interval would be extremely short for rabies, would it not?

DR. HARRIS: In general I would agree with you. I have seen one case of rabies in a child bitten on the finger thirteen days earlier. That child, interestingly enough, also had mumps, so perhaps infection with one virus reinforced the other. In should also be mentioned that Marinesco observed the occurrence of encephalomyelitis in two patients bitten by a dog which, under observation, was not found to be rabid. Neither patient was treated, but in the fourth week one patient developed encephalomyelitis and in the sixth week the other one did. A virus was isolated from both of these patients which killed rabbits in three days. The virus obviously was not that of rabies; it is therefore conceivable that "paralytic rabies" may indeed be due to another virus.

DR. ALEXANDER: In summary I believe we would agree that this patient probably died of encephalomyelitis secondary to antirabies therapy.

Clinical Diagnosis: Encephalomyelitis due to antirabies vaccine.

PATHOLOGIC DISCUSSION

DR. FRED T. CALDWELL: The gross anatomic findings were remarkable only for the presence of thick mucopurulent exudate in the trachea and bronchi and diffuse congestion of the leptomeninges.

DR. DAVID E. SMITH: Material was taken at autopsy from the spinal cord and the hippocampus and inoculated intracerebrally into two groups of mice. All these animals survived. Furthermore, the histologic examination of the nervous system failed to detect inclusion bodies. There was, therefore, no evidence whatsoever that this patient died as a direct effect of viable rabies virus.

Sections of the viscera failed to show any significant pathologic lesions, and morphologic evidence of this patient's fatal disease was found only after extensive histologic investigation of the peripheral nerves and central nervous system. The lesions present were widely disseminated and relatively small. Figure 1 illustrates perivascular infiltration of lymphocytes in a nerve root of the cauda equina. Scattered lesions of this type were found in other roots of the cauda equina, nerves of the lumbosacral plexus, an intercostal nerve and two cranial nerves. In other nerves there were spaces as illustrated in Figure 2. These spaces between nerve bundles could very easily have been artifacts, except that they contained a granular eosinophilic precipitate. Because of their number, distribution and content of precipitated protein they were interpreted as evidence of actual edema within the nerve. Figure 3 shows an almost constant reaction that was present in all the sections prepared of the spinal ganglia. Many of the neurons of the dorsal ganglia had pyknotic nuclei and were surrounded by a wide clear space between the nerve cell and the pericellular sheath. That clear space also could have been artifactual; but because it was so wide and so constant in that particular element of these specimens, it very likely represented a swelling and rarefaction of the cytoplasm of the nerve cell with a consequent unusual degree of shrinkage under the influence of the fixing and staining reagents. The most significant finding, however, was the increased number of satellite cells about all the ganglion cells. A normal section contains only about six such cells around each neuron, while in this preparation there were a great many more in nearly every instance. Figure 4 shows a reaction that was identified in several sites, principally in the white matter of the spinal cord in the thoracic and lumbar regions, but also in some of the spinal nerves as they crossed the ganglia. There were many axon cylinders that were thicker than are normally seen in preparations of the spinal cord, but most significantly they were tortuous and folded acutely at many points along their course. These changes constituted clear evidence of acute reaction and swelling in the axon processes themselves.

No lesions were found in the brain except for a very few foci of questionably increased numbers of microglia about some small blood vessels. Numerous stains for myelin were made of peripheral nerves and of the central nervous system; none of them contained significant changes. Fat stains were made in an attempt to detect the products of the breakdown of myelin without any positive findings. The perivascular zones were examined particularly carefully and the myelin cylinders were found to be preserved right up to the walls of the blood vessels. There was a suggestive slight accentuation of perivascular spaces and edema in many of the septa

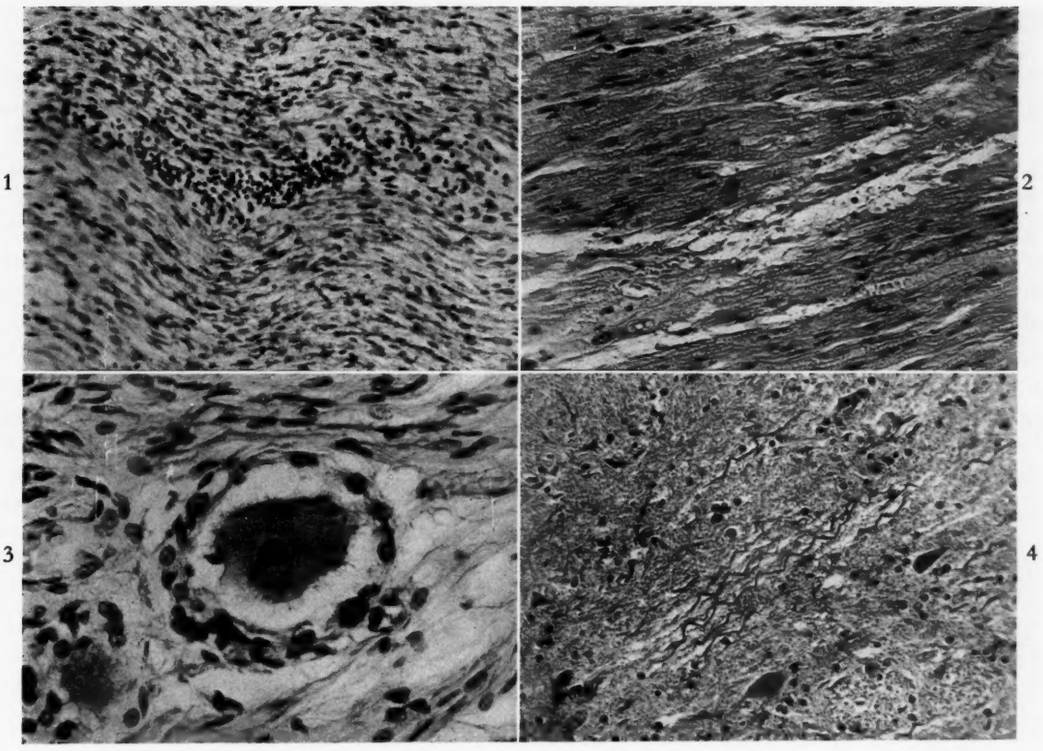

FIG. 1. Perivascular infiltrate of lymphocytes in a root of the cauda equina. No pathologic changes in the myelin sheaths of this or other nerves were detected.
FIG. 2. Interfasicular edema with precipitated protein in an intercostal nerve.
FIG. 3. Increased numbers of satellite cells and a wide subcapsular space about a typical neuron in a dorsal spinal ganglion. The enlarged space is possibly due to shrinkage of an abnormally swollen and rarefied cytoplasm.
FIG. 4. Thickened and tortuous axon cylinders from the grey matter of a section of the spinal cord. This change was present at many levels of the cord.

of the spinal cord, which were possibly of significance. There was one focus of petechiae in the lumbar spinal cord which was the only evidence of hemorrhage.

The pathologic findings suggested that this was a disease that affected particularly the peripheral nerves and axons of the central nervous system. The cell bodies of the central nervous system contained no significant morphologic changes, but the axons of these cells were swollen, there were slight lymphocytic infiltrates about vessels in the peripheral nerves and the satellite cells of the spinal ganglia were increased in number. Changes such as these are typically found in sporadic fatal cases of the Landry-Guillain-Barré syndrome. They do not suggest disseminated encephalomyelitis as is seen after measles or after experimental inoculation of brain substance, although most textbooks mention the post-antirabic vaccinational reactions only to compare them to such lesions.

There have been reported very few fatal cases of reaction following antirabic treatment in which there have been autopsies. Of forty-eight such cases I was able to review a histologic picture of acute myelitis or encephalomyelitis was described in thirty with prominent perivascular infiltrates in the central nervous system. Peculiarly and unlike the disseminated encephalomyelitis after measles or the inoculation of brain substance, these were predominately lesions of the spinal cord with relatively minor alterations in the cerebrum in nearly all the cases. There are at least four cases,[5,6] however, in which there was evidence of acute degeneration in the neurons or axons of the

[5] BASSOE, P. and GRINKER, R. R. Human rabies and rabies vaccine encephalomyelitis. *Arch. Neurol. & Psychiat.* 23: 1138–1160, 1930.

[6] GETZOWA, S., STUART, G. and KRIKORIAN, K. S. Pathologic changes observed in paralysis of the Landry type: a contribution to the histology of neuroparalytic accidents complicating antirabic treatment. *J. Path. & Bact.* 37: 483–500, 1933.

central nervous system and inflammation only in the peripheral nerves.

From the reported cases it is apparent that most fatal reactions to antirabic vaccination are of the nature of a disseminated encephalomyelitis that very possibly represents an allergic phenomenon; but a few, like the present case, have a morphologic picture of an acute polyradiculoneuronitis. The latter disease in its sporadic form is of unknown etiology although it is suspected of being due to a virus. It is futile to speculate as to whether such a virus might be introduced by the original animal bite that prompted vaccination, or in the vaccine, or might be coincidently acquired; but there is no evidence that it is a recognizable form of the rabies virus. On the other hand, the reaction in the tissues is so slight and essentially non-specific that it might represent a response to some at present unsuspected causal agent.

Anatomic Diagnoses: Reaction to antirabic vaccination manifested by perivascular infiltrates of lymphocytes in peripheral nerves, interstitial edema in nerves and thickened and convoluted axons in the spinal cord and spinal ganglia; petechiae in the lumbar spinal cord; acute mucopurulent tracheobronchitis.

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Special Feature

Southern Society for Clinical Research

ABSTRACTS OF PAPERS PRESENTED AT THE FIFTH ANNUAL MEETING,
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SPATIAL VECTORCARDIOGRAM IN RIGHT BUNDLE BRANCH BLOCK. *J. A. Abildskov (by invitation), C. E. Jackson (by invitation), G. E. Burch and J. A. Cronvich (by invitation).* (From the Department of Medicine, Tulane University School of Medicine, New Orleans, La.)

Theoretic considerations and limited actual experience indicate that the spatial vectorcardiogram may provide information not contained in the electrocardiogram as currently used. This report concerns findings in right bundle branch block which suggest that the recognition of ventricular hypertrophy may be facilitated by this method. Projections of the vectorcardiogram on planes of the equilateral tetrahedral reference system have been recorded and used to construct models representing the three-dimensional vectorcardiogram. In some instances two plane projections which can be viewed stereoscopically were also obtained.

Twenty-four subjects have been studied. Clinical and roentgenographic evidence of left ventricular hypertrophy was present in eighteen subjects although the electrocardiograms of only five indicated such enlargement. The QRS sE-loops of seventeen of these subjects were similar, having smooth contours with axes in the first or second sextants of the triaxial reference system in the frontal plane and directed somewhat posteriorly. Records from three subjects with right ventricular hypertrophy were characterized by having the major portion of the frontal plane projection situated to the right of the isoelectric point. The initial portions of the records of three subjects without cardiac enlargement were similar to those of normal subjects.

EFFECT OF INTRAVENOUS DIGITOXIN ON FLUID DISTRIBUTION IN HOSPITALIZED MALES WITHOUT CARDIOVASCULAR DISEASE. *Jerry K. Aikawa, Victor H. Knight and Malcolm P. Tyor (introduced by George T. Harrell).* (From the Department of Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.)

Although the actions of digitalis on the heart are well known, its effect on the distribution of body fluids in normal individuals has not been reported. Because the cardiac glycosides are similar in chemical structure to other cyclopentenophenanthrene derivatives which are known to affect salt and water metabolism—for example, the sex hormones and the adrenal cortical hormones—they might be expected to produce some alteration in the distribution of fluids in the body. The present study was conducted in order to determine the effect of intravenous digitoxin on the plasma and extracellular fluid spaces in normal individuals.

Serial determinations of the plasma volume by the T-1824 dye method, the extracellular fluid by the thiocyanate method and the serum protein by the biuret method were performed in five hospitalized males without cardiovascular disease. When 1.2 mg. of digitoxin was administered intravenously, a significant decrease in the serum protein concentration and an increase in the total circulating protein content was found to occur at eight hours. The results were interpreted as indicating a shift in water into the vascular compartment.

STUDIES IN LIVER PHYSIOLOGY. VII. THE ISOLATED RAT LIVER CAPABLE OF SUSTAINED BILE PRODUCTION. *Ralph W. Brauer and (by invitation) Rita L. Pessotti and Philip Pizzolato.* (From the Department of Pharmacology and Experimental Therapeutics and the Department of Pathology, Louisiana State University School of Medicine and the Veterans Administration Hospital, New Orleans, La.)

A preparation will be described which permits maintenance of a completely isolated rat liver in an artificial circulation under such conditions that sustained bile production continues for twenty-six or more hours. Comparison of this preparation with the normal liver *in situ* shows that in many respects normal or near normal function is retained. Possible application of such a preparation to various problems in liver physi-

ology and liver pathology will be discussed, including the effects of various anesthetics, the action of agents alleged to block the reticuloendothelial system and the handling of dyes like bromsulphthalein. A somewhat more detailed description will be presented of results bearing on the mechanism of bile secretion.

EVALUATION OF PERIPHERAL BLOOD FLOW FROM THE RATE OF CLEARANCE OF LOCALLY INJECTED Na^{24} *C. Erick Bell, Jr. (by invitation) and Harold D. Green.* (From the Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.)

Intramuscular blood flow was estimated by measuring the rate of uptake of Na^{24} injected into the gastrocnemius muscle. The K values (an expression of the clearance of Na^{24}) for normals and patients with peripheral vascular disease so overlapped in the range of 0.03 to 0.05 that no definite conclusions could be reached despite a significant difference in the mean values. Because of this overlapping in the resting state we decided to determine the flow rate in patients and normals under conditions approaching maximum flow. As a means of increasing the flow we used six minutes of alternate plantar and dorsiflexion of the foot. The uptake of Na^{24} was measured during a six-minute resting period prior to exercise and during the exercise. The K values for the normals averaged 0.043 during the resting period and 0.101 during the exercise. For the patients the resting values averaged 0.021 and during exercise 0.046. Following the exercise the K values approximated those of the resting period. Hyaluronidase injected with the Na^{24} did not cause any significant change in the K values.

The uptake of Na^{24} is a measurement of intramuscular blood flow. The severity of peripheral vascular disease can be estimated by measuring the clearance of Na^{24} during exercise.

INTRAVENOUS INJECTION OF DEXTRAN FOR THE EXPANSION OF PLASMA VOLUME. *Walter Lyon Bloom (introduced by James V. Warren).* (From the Departments of Biochemistry and Medicine, Emory University, School of Medicine, Atlanta, Georgia, and Research Division, Lawson Veterans Administration Hospital, Chamblee, Ga.)

Although dextran has been accepted clinically as a "blood substitute" in Sweden and in England, this polysaccharide has not been used extensively in America because of several adverse reports of reactions following its intravenous injection. This report presents data on the intravenous use of a partially hydrolyzed dextran produced in this country. Fifty convalescent patients have received 500 cc. of 6 per cent dextran intravenously. No reactions have been observed during or after injection. Clinical tests of renal function (urinalysis, PSP, creatinine clearance, NPN) and hepatic function (BSP, serum proteins, cephalin flocculation) showed no evidence of functional alteration following dextran injection.

Studies of dextran in the blood and urine by a method of the author indicated that dextran remained in the blood for as long as five days and from 30 to 50 per cent of the injected dextran was excreted in the urine. Hematocrit and serum protein determinations indicated definite hemodilution which persisted for twenty-four hours. The data indicate that this partially hydrolyzed polysaccharide is satisfactory for the osmotic expansion of plasma volume and unassociated with reactions or measurable impairment of renal or hepatic function.

RENAL EXCRETION OF HYPERTONIC SODIUM SOLUTIONS IN ADDISON'S DISEASE WITH OBSERVATIONS ON THE EFFECTS OF CORTISONE. *C. H. Burnett, A. S. Relman, B. A. Burrows, J. H. Sisson and S. H. Ingbar (introduced by Carl A. Moyer).* (From the Evans Memorial Hospital, Boston University of Medicine, Thorndike Memorial Laboratory, Harvard Medical School, Boston, Massachusetts, and Southwestern Medical College, Dallas, Tex.)

The previously described delayed renal excretion of sodium in adrenal insufficiency following administration of excessive salt was studied further. The responses to administration of hypertonic saline intravenously of five patients with Addison's disease were compared with those of six normal subjects. The observations indicate that the renal defect was the result of inability rapidly or significantly to increase either urine sodium concentration or urine flow. Although simultaneous inulin clearance varied in different patients from very low to normal values, there was no correlation with the quantity of sodium filtered and the delayed excretion of sodium nor was it possible to demonstrate an increased tubular reabsorption of sodium. In two patients removal of sodium administered as hypertonic sodium para-aminohippurate was much more prompt than it was following hypertonic saline. Cortisone improved ability to ex-

crete water excesses rather promptly, but improvement in salt excretion during treatment with this hormone was much less uniform. Potassium excretion was unaffected and chloride excretion roughly paralleled that of sodium. It is suggested that the renal defects in adrenal insufficiency reflect impaired responsiveness of the renal tubule to demands to increase or decrease salt excretion and to increase water excretion.

EFFECTS OF STRESS AND ACTH ON THE PLASMA AMINO ACID NITROGEN LEVELS OF HUMAN SUBJECTS. *Thomas W. Burns (by invitation), Wilmer J. Coggins (by invitation), Albert Heyman and Frank L. Engel.* (From the Departments of Medicine, Duke University School of Medicine, Durham, North Carolina and Emory University School of Medicine, Atlanta, Ga.)

Increasing evidence is being accumulated to cast doubt on the concept that all of the characteristic alterations in nitrogen metabolism associated with non-specific stress are a direct consequence of stimulation of the adrenal cortex. Here an instance is demonstrated in which the results of increased cortical secretion on one hand and stress on the other are at variance.

The plasma amino acid nitrogen (AAN) levels of twelve subjects were found to be constant over a six-hour period. Seven subjects injected intramuscularly with 40 mg. of ACTH (Wilson's corticotrophin) failed to manifest any alteration in their AAN during the subsequent eight hours but showed a significant eosinopenia. However, in nine individuals receiving ACTH for chronic illnesses there was a significant elevation during the third to tenth day of treatment.

A marked fall in AAN levels followed the intravenous administration of typhoid vaccine as has been reported with other types of stress. This fall occurred even when the fever response was obliterated with aminopyrine and in both cases was associated with a fall in eosinophils. Pretreatment with 40 mg. of ACTH four hours before typhoid administration largely abolished the characteristic fall in AAN. The significance of these results will be discussed.

STUDIES ON THE EFFECTS OF ETHYL CHLORIDE ON CARDIAC RHYTHM IN DOGS. *Oliver F. Bush (by invitation) and John Adriani.* (From the Department of Anesthesia, Charity Hospital, New Orleans, La.)

Ethyl chloride is still used as an inhalation anesthetic for minor surgical procedures or as an induction agent for other anesthetics. Fatalities occur from time to time, usually during induction. The exact etiology of these fatalities has never been defined although it is believed that they are due to cardiac failure. Data on the pharmacologic effects of ethyl chloride are meager. It was decided, therefore, to investigate some of its action on the circulatory system. Dogs were used for this study. Anesthesia was induced by the open technic. The dogs were intubated and anesthesia was then carried on, using a closed rebreathing system. Continuous electrocardiographic tracings, using lead II, were taken throughout each experiment.

Two separate effects on the heart were noted. The first, which occurred shortly after induction, resulted in A-V block, extreme bradycardia and, in some cases, ventricular fibrillation. This effect appeared to be due to vagal stimulation and could be prevented by atropine or atropine and morphine. The second effect, which occurred later in the course of anesthesia, appeared to be due to a direct depressant action of the drug on the cardiac tissues. This was manifested by a marked tachycardia which, if allowed to proceed, terminated in asystole or ventricular fibrillation. Premedication with atropine did not protect against this type of response.

INDUCED VARIATIONS IN GLOMERULAR FILTRATION RATE AND THE URINARY EXCRETION OF WATER AND CHLORIDE. *Walter H. Cargill and (by invitation) Sid Sellers and Merton Shure.* (From the Department of Physiology, Emory University School of Medicine, Atlanta, Ga.)

In an attempt to correlate variations in glomerular filtration rate (endogenous creatinine clearance), urine flow and chloride excretion, these functions were measured in resting subjects and under the following conditions: standing in air, standing immersed in water to heart level and lying with pressure applied to the abdomen and thighs. It was found that immersion not only prevented the fall in filtration rate, urine flow and chloride excretion which occurred while standing in air but also actually produced a definite increase in all three functions. Filtration rate, water and chloride excretion diminished when pressure cuffs on the upper thighs were inflated, and decreased still further when abdominal compression was added, suggesting an effect of abdominal pressure other than simple pooling of blood in the lower extremities. Although changes in the three activities measured were always in the same directions under these various conditions, no quantitative correlation

between glomerular filtration and either urine flow or chloride excretion could be obtained. At low rates of flow an apparent correlation exists between water and chloride excretion. This is believed to be indicative of posterior pituitary activity. Evidence obtained by the administration of pitressin and hypertonic saline solution will be presented to support this concept.

RELATIONSHIP OF VITAL CAPACITY TO PULMONARY BLOOD VOLUME. *Walter S. Coe (by invitation), Maurice M. Best (by invitation) and Hampden C. Lawson.* (From the University of Louisville Institute for Medical Research, Louisville, Ky.)

The quantity of blood contained in the pulmonary vessels, left heart, aorta and certain larger arteries can be calculated according to the formula of Stewart when the cardiac output and mean circulation time are known. Comparative data on calculated pulmonary blood volume in the normal human subject have been obtained by making two dye (T-1824) injections in each subject: into an antecubital vein and into the right auricle or ventricle. The vein injections yielded calculated volumes larger than the intracardiac injections. The difference in volumes is greatly in excess of the volume of blood standing between the heart and the site of vein injection.

Data have been obtained on normal human subjects using intracardiac dye injections which show the effect of postural changes on the calculated pulmonary blood volume. In general there is a decrease in volume when the subject is tilted feet-down, a decrease of the same order of magnitude as the increase in vital capacity. Data on pulmonary circulation time suggest that the reduction in cardiac output in the feet-down posture is associated with an equivalent reduction in the cross-sectional area of the stream bed, mean velocity of flow remaining unchanged.

INFLUENCE OF DIET AND LIPOTROPIC AGENTS ON THE PHOSPHOLIPIDE TURNOVER IN PLASMA OF NORMAL HUMAN ADULTS. *W. E. Cornatzer, David Cayer and William A. Lambeth (by invitation).* (From the Departments of Biochemistry and Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.)

The formation of phospholipides is a fundamental function of the liver. Previous study has indicated a close parallelism between the histologic finding of fat in the liver as shown by needle aspiration biopsy and the response in the plasma phospholipide turnover after a large dose of lipotropic material as studied with radiophosphorus. The time interval for the development of fatty infiltration of the liver is well established in experimental animals but unknown in humans. The use of radiophosphorus as an indicator offers an opportunity to determine the time required in normal subjects given a low protein diet for the development of sufficient alteration in fat metabolism which can be noted by a significant change in phospholipide synthesis.

Twelve normal adults maintained on experimental diets, low in protein, (7.6 per cent) were studied. In three of the subjects a slight increase in phospholipide synthesis occurred after the administration of a single large dose of lipotropic agents. The time interval that is required to produce a response to lipotropic agents in most subjects is greater than nine days.

ASSOCIATION BETWEEN PRIMARY AMINES AND PRESSOR SUBSTANCES IN URINE. *D. F. Davies, H. Miller and Henry A. Schroeder.* (From the Hypertension Division, Department of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, St. Louis, Mo.)

Irrefutable evidence has been reported from this laboratory for the existence of an organic base (pherentasin) in human hypertensive and not in normal blood, which gives a pressor response in the rat, and which is probably a primary amine. A study of pressor amines in urine was indicated because of the probability of a renal defect in amino acid metabolism and the paucity of specific data on urinary pressor substances.

Fractional chromatography on silica gel was carried out on twenty-four urine samples. One ml. of each eluate fraction was tested intravenously in rats for pressor or depressor activity. The remaining portion of each eluate was extracted with chloroform from alkaline solution, evaporated almost to dryness and chromatographed on Whatman No. 1 filter paper sheets using a butanol-water solvent. The ninhydrin-sensitive substances were developed by spraying and Rf values calculated for each spot. Good correlation was found to exist between the Rf values of the primary amines separated by chromatography and the blood pressure effect of the eluates. They could not be identified with known primary amines of biologic interest studied previously.

INFLUENCE OF HIGH OXYGEN TENSION ON THE METABOLISM OF PIGEON BONE MARROW. *James S. Dinning, (by invitation) and Horace N. Marvin.* (From the Departments of Biochemistry and Anatomy, University of Arkansas School of Medicine, Little Rock, Ark.)

Pigeons were placed in a box through which was circulated pure therapy oxygen at a rate of 2 to 4 L. per minute for sixteen hours a day. Birds were removed at weekly intervals for four weeks and complete blood counts were made. The birds were killed by decapitation and the bone marrow was removed and suspended in saline. Endogenous oxygen consumption, choline oxidase and succinoxidase were determined by the Warburg technic. Similar determinations were made on control birds.

It was found that the high oxygen tension decreased the hematocrit, hemoglobin concentration and red cell counts of the peripheral blood. Peripheral leukocytes were not consistently affected. Endogenous oxygen consumption and succinoxidase activity of the bone marrow were the same in the control and experimental groups; however, the high oxygen tension reduced bone marrow choline oxidase to approximately one-third of the control values.

As estimated from cell counts of the suspended marrow, there was no effect of high oxygen tension on the total number of the bone marrow cells.

COMPARISON OF ADRENOLYTIC BLOCKING EFFECTS OF REGITINE (C-7337) AND BENODAINE. *J. R. Emlet (by invitation), K. S. Grimson and (by invitation) B. H. Metcalf.* (From the Department of Surgery, Duke University School of Medicine, Durham, N. C.)

Our observations that small amounts (1/12 to 1/3 mg./kg.) of regitine (C-7337) intravenously or intramuscularly reduced blood pressure before and during operation in four patients with pheochromocytoma stimulated this comparative study. The effect of single doses of regitine of 5 mg. and twenty minutes later 10 mg. per patient intramuscularly was compared with that of benodaine 15 mg. intravenously in forty usual hypertensive patients. Less variation of pressure and fewer side effects followed regitine. In addition to the four patients with pheochromocytoma a reduction of blood pressure also occurred in four patients with uremia.

The adrenolytic blocking actions of regitine and benodaine against the effects of injected equipressor doses of levo-epinephrine and levo-arterenol were studied. Under chloralose anesthesia four dogs were used with regitine and four with benodaine, observing the response to the injected sympathomimetic amines before and after administration of 0.02 mg./kg. to 5 mg./kg. of the adrenolytic agents. Small amounts of regitine or benodaine somewhat reduced the pressor responses, the reduction with L-epinephrine being more pronounced. Following amounts which effected reversal of L-epinephrine and blocked or markedly reduced the response of L-arterenol, the action of benodaine persisted up to one-half hour and that of regitine for one hour or more.

PRESSURE-VOLUME RELATIONSHIPS IN THE PULMONARY CIRCUIT OF MAN. *E. Harvey Estes, Jr. (by invitation), Joseph S. Wilson (by invitation), Joseph T. Doyle (by invitation) and James V. Warren.* (From the Departments of Physiology and Medicine, Emory University School of Medicine, and Grady Memorial Hospital, Atlanta, Ga.)

Recent technical advances have made possible an analysis of the pressure-volume relationships in the pulmonary circuit of man. In thirty-four normal individuals studied under relatively basal conditions a linear relationship was found between the pulmonary arterial pressure and the blood volume. In twelve subjects rapid intravenous infusions produced changes in blood volume and pulmonary pressure which followed remarkably the line of relationship derived from data in the normal resting subjects. In five other individuals subjected to blood loss of 500 to 900 cc. the pressure-volume relationship also followed the line of regression obtained from normal subjects. In other situations, such as hypoxia, hyperthyroidism and certain types of cardiac failure, a deviation from the linear relationship was found. This indicates either vasomotor activity by the pulmonary vessels or redistribution of blood with a relatively increased amount in the pulmonary circuit.

These observations demonstrate that the amount of blood contained within the vascular system is an important factor in determining the pulmonary vascular pressures. Furthermore, under the circumstances studied increasing or decreasing the amount of blood in the vascular system does not alter the ratio of blood contained within the pulmonary circuit. This is evidence against the alleged "reservoir" function of the pulmonary vascular bed.

CLINICAL EVALUATION OF HEXAMETHONIUM, A NEW GANGLIONIC BLOCKING AGENT—PRELIMINARY REPORT. *Edward D. Freis and (by invitation) Harold W. Schnaper, Robert L. Johnson, John C. Rose and Frank A. Finnerty, Jr.* (From the Georgetown University School of Medicine, Department of Medicine, Washington, D. C.)

Previous studies in this laboratory demonstrated that following hexamethonium (C6), a new ganglionic blocking agent, the increase in blood flow in the toes usually was greater and more prolonged than after either priscoline or tetraethylammonium.

In ten normal subjects the average increase in foot blood flow (plethysmograph) was as great after 50 to 100 mg. C6 intravenously as after epidural and/or spinal anesthesia using procaine. Because of the apparent completeness of sympathetic blockade a clinical trial of the drug seemed indicated.

In severe hypertension only three of ten patients maintained significant hypotensive responses after one month of continuous therapy. Two of these cases, however, have shown regression of the malignant phase. In six cases of acute thrombophlebitis rapid subsidence of symptoms and signs occurred in every case. In arteriosclerosis and thromboangiitis obliterans hexamethonium appeared to be useful in evaluation of the degree of vasospasm and in the treatment of acute complications. In causalgia C6 produced relief for variable periods in nine of ten cases and was of value in selection of cases for sympathectomy. Additional clinical advantage of C6 are (1) comparative lack of side effects and low toxicity, (2) duration of action of at least several hours and (3) effectiveness by all routes of administration.

ADRENAL RESPONSE TO ACTH ADMINISTRATION IN VARIOUS CLINICAL CONDITIONS. *Douglas Gordon (by invitation), Benjamin Horwitt (by invitation) and Albert Segaloff.* (From the Department of Medicine, Tulane University and Alton Ochsner Medical Foundation, New Orleans, La.)

ACTH has been administered over a forty-eight-hour period as a means of evaluating adrenal function, utilizing a modification of the method of Forsham and Thorn. A decrease in total blood eosinophils and increases in urinary excretion of 17-ketosteroids, chemical cortins and uric acid/creatinine ratios have been selected as criteria for adrenal responses. These changes were measured at intervals after ACTH administration (25 mg. initially followed by 10 mg. every 6 hours, × 7).

These ACTH tests have been carried out in a variety of clinical conditions. The increased ketosteroid excretion four hours after ACTH administration has been the most consistent change noted while the uric acid/creatinine changes have been the least consistent. As anticipated, patients with Addison's disease showed no significant changes following ACTH administration. Elderly patients with advanced carcinoma of the breast showed insignificant changes in ketosteroids but good changes in cortins. A similar response was seen in noncancerous chronic illness, as well as in elderly women without cancer. Two patients with pituitary failure showed delayed responses initially with satisfactory responses after forty-eight hours. In thyroid myxedema the control ketosteroid and cortins were low but the response to ACTH was fairly good. One patient with Cushing's syndrome showed marked increases on both chemical cortin and ketosteroid excretion following ACTH. Three months after bilateral hemiadrenalectomy was done a repeat ACTH test gave no significant response.

ELECTRICAL DEFIBRILLATION OF THE HEART THROUGH THE UNOPENED CHEST. *Arthur C. Guyton.* (From the Department of Physiology and Biophysics, University of Mississippi School of Medicine, University, Miss.)

Preliminary studies using electrodes directly on the heart indicated that a train of stimuli for approximately 0.05 to 0.5 seconds is far superior for defibrillation than is a single stimulus, even though the single stimulus may have a voltage fifty times that of the multiple stimuli. It was also shown that powerful thyratron discharges were not superior to 60 cycle alternating current. Also, the larger the electrodes on the heart, the greater was the current through the heart and, consequently, the lower was the voltage necessary for defibrillation.

Applying the principles derived from the open chest experiments, defibrillation apparatus, utilizing an electronic timer circuit and 2 kilowatt transformer, has been developed for use through the unopened chest. The electrodes are applied anteriorly and posteriorly. When using 300 volts at approximately 20 amperes for 0.1 second, defibrillation has thus far been universally successful in several hundred trials in dogs. Several dogs have been defibrillated more

than thirty times—one fifty times—and have no evidence of residual cardiac, neurologic or other damage.

On the basis of these experiments apparatus which should be practical has been designed for defibrillation of the human heart without opening the chest.

SPECTROPHOTOMETRIC OXYGEN DETERMINATION IN SMALL SAMPLES OF WHOLE BLOOD. APPARENT INCREASE IN OXYHEMOGLOBIN UNDER HIGH OXYGEN TENSIONS. *J. B. Hickam and (by invitation) R. Frayser.* (From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

A new, convenient and simple spectrophotometric method is described for the determination of arteriovenous oxygen differences in whole blood samples. The method uses the Beckman DU spectrophotometer equipped with cuvettes having a short light path (approximately 1.6 mm.). The method gives results which agree well with gasometrically determined oxygen differences. For oxygen differences ranging between 0.65 and 10.00 volume per cent in fifty-four pairs of blood samples analyzed by both methods, the spectrophotometric results conformed to the relation: oxygen difference = 13.08 by optical density difference (standard deviation less than 0.2 volume per cent). The absolute value of oxygen content can be estimated.

Using this method a study has been made of the effect of high oxygen tensions on whole blood equilibrated in tonometers at 38 degrees under approximately 35 mm.pCO_2. The results suggest that hemoglobin is not entirely saturated with oxygen at 150 mm.pO_2. Optical density changes are obtained which would correspond, by the formula given before, to an average increase of 2.4 per cent saturation in proceeding from 150 to 630 mm.pO_2. Even though the formula may not apply well in this high tension range, a definite progressive change occurs in the direction of greater saturation. This is reversible on decreasing the oxygen tension. Similar observations have been made on hemolyzed blood equilibrated at room temperature.

INFLUENCE OF THE POSTERIOR PITUITARY ON SODIUM AND WATER EXCRETION. *Bernard C. Holland (introduced by Eugene A. Stead, Jr.).* (From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

Data have been presented by Murphy to show that small doses of pitressin and endogenously produced antidiuretic hormones of the posterior pituitary gland have the same effect on the excretion of water, sodium and chloride. In observations lasting up to one hour they cause a striking retention of water but no change in the excretion of Na+ and Cl−. These data might be interpreted as showing either that water retention does not cause Na+ and Cl− retention or that the mechanism for Na+ and Cl− retention secondary to water retention is a sluggish one requiring longer than one hour to become effective. Normal subjects were given 200 cc. of 0.2 per cent NaCl orally every hour for twenty-four hours. Urine was collected every hour for electrolyte determinations. These subjects had an almost continuous diuresis with the excretion of 86 per cent of the administered water and 63 per cent of the administered sodium. Four days later, in addition to the hypotonic saline, they received every hour an intravenous injection of one milliunit of pitressin per kilo of body weight. These subjects gained in weight and excreted only 14 per cent of the ingested water. The effect of the retention of water on the excretion of sodium and chloride will be presented.

PLASTIC CARDIAC VALVULAR PROSTHESES. *Charles A. Hufnagel (introduced by Hugh Hussey).* (From the Laboratory for Surgical Research, Georgetown University School of Medicine, Washington, D. C.)

In previous publications it has been shown that certain plastic tubes can function adequately as replacement of segments of the thoracic aorta. From that work a plastic valve has been developed which functions well for long periods of time in all spatial relations without any clotting and under all physiologic pressures. The method of use of this type prosthesis as aortic and as auriculoventricular valves is presented. Technics of fixation are discussed. Several plastic materials useful in construction of such valves are compared.

The results of these methods in twenty animals who have been studied prior to and for one month to two years after resection of the normal aortic valve and insertion of the valvular prosthesis are given.

RESPONSE OF THE PERIPHERAL BLOOD AND BONE MARROW TO LOCALIZED X-RAY THERAPY IN CARCINOMA OF THE CERVIX. *Lucile W. Hutaff and Helen W. Belding (introduced by George T. Harrell).* (From the Department of Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.)

Ten consecutive cases of carcinoma of the cervix were studied because of the previous observation of leukopenia following x-ray therapy. Initial bone marrow aspirations were obtained from the iliac crest and samples of the marrow from both the iliac crest and sternum were removed at intervals varying from seventeen to sixty days after the beginning of approximately 2,400 r units to each of four fields in the pelvic region. Daily studies of the white blood count and differential count were made during the period of x-ray therapy.

A moderate decrease in total white blood cell count at levels of 2,800 to 4,000 per cu. mm. was observed during the period of treatment. This decrease was most marked within the first five days but was quite variable. The appearance of small numbers of metamyelocytes occurred during the seventh and eleventh day after therapy began. There was a pronounced change in the cellularity of the iliac marrow with reduction in young myeloid and erythroid cells. The sternal marrow by contrast exhibited cellularity increased above normal in all marrow elements, with a moderate shift to the right in the myeloid series.

TREATMENT OF DICUMAROL-INDUCED HEMORRHAGE WITH VITAMIN K_1 OXIDE. *David F. James, Thomas D. Johnson and Sue Burnett (introduced by Paul B. Beeson)*. (From the Department of Medicine, Emory University Medical School and Emory University Hospital, Atlanta, Ga.)

Vitamin K_1 oxide, already known to be highly effective in combating hypoprothrombinemia induced by dicumarol, is herein shown to be efficacious in producing cessation of hemorrhage from dicumarol. The present report deals with a series of twelve patients studied in the past three years. Each of these was hemorrhaging as a consequence of the action of dicumarol. The gastrointestinal, genitourinary, subarachnoid and subcutaneous regions were involved. Some of them had been given other materials known to have vitamin K activity in various doses, ranging from 62 to 240 mg. This was combined in certain cases with blood transfusions of from 500 cc. to 2,000 cc. during the same period. Our observations confirmed the already widely recognized fact of the inadequacy of these materials and methods of treating dicumarol-induced bleeding.

Each of these patients was given 500 mg. of vitamin K_1 oxide in a single intravenous dose. Although the persistence of the results of previous bleeding (e.g., guaiac-positive feces) at times rendered impossible an accurate estimate of the exact time of cessation of bleeding, examination of each record reveals slackening of bleeding which in every case but one is inconsistent with the natural history of bleeding from dicumarol intoxication and was doubtless brought about through the agency of vitamin K_1 oxide. The prothrombin times, in most instances markedly prolonged, shortened *pari passu* with cessation of bleeding, returning to normal levels in an average of 7.6 hours.

RESISTANCE OF THE NORMAL THYROID TO 6-N-PROPYLTHIOURACIL. *Edwin C. Jungck, Willis E. Brown, C. G. Sutherland and G. W. Jackson (introduced by Paul L. Day)*. (From the Departments of Obstetrics and Gynecology and Physiology, University of Arkansas School of Medicine, and The Arkansas State Hospital for Nervous Diseases, Little Rock, Ark.)

In an attempt to induce hypothyroidism and menstrual irregularities a group of eight normally menstruating women each received orally over a period of eight months 6-n-propylthiouracil in increasing doses of 250 mg. to 3,000 mg. daily. The drug was administered in two or three divided doses daily. They received 3,000 mg. daily for the final eight weeks' period. The observations made were as follows: Biweekly red blood count and white blood count, weekly basal metabolic determinations and endometrial biopsies, and constant clinical observation. One of us personally supervised the administration of the drug.

During the total period of observation only three anovulatory cycles occurred which were interpreted as a normal occurrence. There were no irregularities of menstruation. No changes were noted in the blood studies even with the high dosages used at the end of the period. The basal metabolic rate showed a tendency to fall slightly but no significant changes were noted. No clinical evidence of hypothyroidism and no enlargement of the thyroid was noted.

CLINICAL AND METABOLIC OBSERVATIONS IN THE DE TONI-FANCONI SYNDROME. *Laurence H. Kyle, Paul D. Doolan and William P. Walsh (introduced by Harold J. Jeghers)*. (From the Department of Medicine, Georgetown University School of Medicine and the Georgetown University Hospital, Washington, D. C.)

Malacic bone disease in association with renal glycosuria, amino-aciduria, hyperphosphaturia

and increased excretion of organic acids appears to be an infrequent but distinct entity known best as the Fanconi syndrome. This bizarre combination of metabolic disorders is of especial interest in that investigation of the abnormalities contributes to basic understanding of bone metabolism, acid-base balance, renal reabsorbtive mechanisms and amino-acid metabolism. This report deals with a portion of the metabolic studies of three patients with the Fanconi syndrome. Two of the patients, a fifteen year old girl and a forty-two year old man, had severe osteomalacia with pseudofractures. By means of paper chromatography a markedly abnormal urinary amino-acid pattern was demonstrated. Both patients showed renal glycosuria and increased excretion of phosphorus and organic acids. The third patient demonstrated all of the urinary abnormalities associated with the Fanconi syndrome but had no evidence of bone disease. Although most instances of the Fanconi syndrome have been seen with rickets, it should be emphasized that this condition may be associated with adult osteomalacia. The disorder must be considered in all individuals with renal glycosuria and patients with either resistent rickets or osteomalacia, especially if glycosuric, should be examined for the presence of abnormal urinary amino acids. Paper chromatography offers a simple but accurate technic for the demonstration of abnormal amino-acid excretion.

A portion of the metabolic studies conducted on these patients will be shown and as time permits various theories relative to the causative mechanism of the bone disease will be evaluated in the light of the results obtained.

EXTRACELLULAR WATER CONTENT OF THE HEART IN RATS SUBJECTED TO ANOXIA MEASURED WITH THE RADIOACTIVE ISOTOPE OF SODIUM. *Janet M. Lemley (by invitation) and George R. Meneely.* (From the Radioisotope Unit of Thayer Veterans Administration Hospital and the Departments of Biochemistry and Medicine, Vanderbilt University School of Medicine, Nashville, Tenn.)

The ventricular myocardium of rats subjected to severe acute anoxia appears on gross examination to be edematous. To put this observation on a quantitative basis the method of Manery and Bale was employed to measure the extracellular water of the myocardium. These experimental animals were exposed under conditions equivalent to an atmosphere 7 per cent oxygen for one hour and killed by exposure equivalent to 3 per cent oxygen. Normal rats of the control group showed an extracellular water content in the myocardium of 28.03 per cent of the wet weight of tissue while in the animals subjected to anoxia the extracellular water in the hearts was 36.29 per cent or 8 per cent higher than that found in normal hearts. The apparent sodium space of hearts from anoxic rats is, therefore, approximately 30 per cent greater than normal and this difference is highly significant (P .001). Skeletal muscle does not exhibit similar changes under anoxia.

Anoxia results in pronounced alterations in the metabolism of the heart. Consideration of this metabolic alteration must take into account the previously described changes. There are also obvious clinical implications of the association of anoxia with myocardial edema, particularly concerning diffusion distances and the nourishment of the myocardial fiber.

ACTIVATION OF DOG SERUM PROFIBRINOLYSIN BY A PURIFIED LUNG PROTEIN AND BY STAPHYLOKINASE. *Jessica H. Lewis and John H. Ferguson.* (From the Department of Physiology, University of North Carolina Medical School, Chapel Hill, N. C.)

In an attempt to understand the physiologic mechanism which results in the occasional appearance in the circulating blood of an active proteolytic enzyme, fibrinolysin, we have studied the *in vitro* activation of its precursor, profibrinolysin. The fibrinolytic enzyme system consists of four components, namely, profibrinolysin, an inactive protein; fibrinolysokinase (s), activator (s) of profibrinolysin; antifibrinolysokinase (s), inhibitor (s) of fibrinolysokinase activity; and antifibrinolysin, inhibitor of fibrinolysin activity. Dog serum profibrinolysin may be activated by at least three types of activators, namely, bacterial, e.g. staphylokinase; tissue, e.g. lung kinase; and serum kinase. Staphylokinase acts as a typical enzyme in the conversion of purified dog profibrinolysin which it completely converts to active fibrinolysin under conditions of optimum pH, ionic concentration and temperature. The staphylokinase concentration determines the rate of fibrinolysin formation but not the final yield which is directly dependent upon the initial prolysin concentration. The activity of lung kinase, a protein fraction derived from the "large granule and microsome" sedimented fraction of homogenated lung tissue, is compared to staphylokinase. Under the trial conditions of pH, ionic concentration and temperature,

lung kinase converted about 60 per cent of the profibrinolysin to lysin. The remainder was apparently converted to inert protein, a reaction which may be similar to the conversion of trypsinogen to trypsin and inert protein at pH 7.0 to 9.0.

EFFECT OF HYPERTHYROIDISM AND MYXEDEMA UPON CEREBRAL BLOOD FLOW AND METABOLISM. *Leonard Madison, Willis Sensenbach and Lamar Ochs (introduced by Arthur Grollman).* (From the Department of Medicine, Southwestern Medical College of the University of Texas, and the Department of Medicine, Veterans Administration Hospital, Dallas, Tex.)

Hyperthyroidism and myxedema afford the opportunity to study the effects of change in total body metabolism upon cerebral metabolism and blood flow. These functions were studied by the nitrous oxide technic in four patients with hyperthyroidism and four patients with myxedema before treatment and when euthyroid. The euthyroid state for each individual was considered the control.

Hyperthyroid patients exhibited an increase in mean cerebral blood flow from 59 to 87 ml. /min./100 gm. brain or 47 per cent. Mean cerebral vascular resistance decreased from 1.55 to 1.00 mm.Hg/ml. blood/100 gm. brain or 36 per cent. Mean cerebral oxygen consumption remained unchanged.

Myxedematous patients revealed a decrease in mean cerebral blood flow from 52 to 46 or 12 per cent. Mean cerebral vascular resistance increased from 1.77 to 2.15 or 21 per cent. No change was noted in mean cerebral oxygen or glucose utilization.

From these data it seems that the brain does not share in the changes in total body metabolism in hyperthyroidism and myxedema. The changes in cerebral blood flow may be related to the alterations in cardiac index reported by others in hyperthyroidism (plus 32 per cent) and myxedema (−47 per cent).

EFFECT OF A GROUP OF BACTERIA AND THEIR CARBOHYDRATES ON HUMAN LEUKOCYTES. *Samuel P. Martin (introduced by E. A. Stead, Jr.).* (From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

The effect of a group of bacteria and their somatic carbohydrates was tested on human leukocytes. The leukocytes were observed in a slide cell, previously described, which allows observation of the oriented migration from a buffy coat. The only materials added were bacteria, their carbohydrates, and a concentration of heparin known to be non-toxic to leukocytes.

Salmonella typhosa and Pseudomonas aeruginosa in concentrations of one to ten bacilli per leukocyte exerted marked to moderate inhibition of migration of the human cells. The purified carbohydrate of these organisms in concentrations of 0.25 to 5.0 gamma per ml. produced the same effect. Micrococcus aureus in the same concentration had no effect on leukocytes.

The somatic carbohydrate of S. typhosa has been noted to exert a negative chemotaxis for guinea pig leukocytes and to prevent diapedesis of leukocytes from blood vessels. The latter effect has been attributed to shock induced by this agent.

The inhibition of migration as tested in this system would seem to indicate a profound effect of these carbohydrates on the leukocyte. It would appear that this effect might be important in the leukopenia as well as the fever and other reactions which are followed with the administration of these materials. In addition to the shock these agents produce it would appear that they have a direct effect on the leukocytes.

THECOMA IN A ONE YEAR OLD GIRL WITH FEMINIZATION. *Horace N. Marvin and (by invitation) Vida H. Gordon.* (From the Departments of Anatomy and Pediatrics, University of Arkansas School of Medicine, Little Rock, Ark.)

The case is that of a one year old girl with the following symptoms: Excessive mammary development, sparse pubic hair, white vaginal discharge, a slightly enlarged uterus, an abdominal mass 4 by 6 cm., precocious bone development, and hyper-irritability and insomnia. Preoperative vaginal smears showed preponderantly cornified epithelial cells. Urinary estrogen determinations were elevated and 17-ketosteroid values were within the normal range.

Upon surgical exploration a tumor of the left ovary 17 by 11 by 9 cm. was found and removed with the left oviduct. Sections of the tumor revealed spindle-shaped cells containing sudanophilic birefringent granules and intercellular reticular fibers. The oviductal epithelium was highly developed and had cilia on its free border. Extracts of the tumor were assayed and found to contain the equivalent of 2 I.U. of estrone per gram of fresh tissue.

Postoperatively the changes were reversed

and the child is now well and normal at the age of four years.

MAXIMUM RATE OF ACETYLATION OF PARA-AMINO-BENZOIC ACID (= "LM-ACETYL-PABA") BY THE LIVER OF THE RABBIT. *Morton F. Mason, Gus Casten (by invitation) and Allan Lindsay (by invitation).* (From the Parkland Hospital and the Department of Internal Medicine, Southwestern Medical School of the University of Texas, Dallas, Tex.)

When the plasma level of PABA was sufficiently raised in rabbits by intermittent administration of the drug by stomach tube, the quantity acetylated per unit time increased to a maximum which was then independent of further increase in plasma concentration of the free drug. This maximum rate for a liver function (Lm) is analogous to a Tm function for the kidney. The acetylaminobenzoate formed was calculated by correcting the quantity excreted in urine (collected by catheter) for a given period by that amount accumulating in the body meanwhile. The latter was estimated, as an approximation, from the change in plasma concentration of acetyl-PABA during the period, assuming a previously determined average apparent volume of distribution equal to 24 per cent of body weight.

Lm-acetyl-PABA, reasonably constant in an individual, exhibited considerable variation in a series of ten male rabbits ranging from 0.39 to 0.89 mg.*/min./kg. body weight. Administration of sodium acetate with PABA (three experiments) did not increase Lm-acetyl-PABA, indicating acetate availability is not a limiting factor determining maximum rate under these conditions.

EVALUATION OF GLUCOSE TOLERANCE IN SEVENTY-ONE SUBJECTS WITH GLYCOSURIA. *John H. Moyer and C. Ray Womack (introduced by James A. Greene).* (From the Department of Medicine, Baylor University College of Medicine, Houston, Tex.)

In an attempt to evaluate variable results obtained when using different glucose tolerance tests the one-dose oral (Standard), the two-dose oral (Exton-Rose), the postprandial and the intravenous tests were performed on a heterogeneous group of seventy-one persons showing glycosuria but no other symptoms of diabetes mellitus. Criteria of normality derived from a similar study (*Am. J. M. Sc.*, 219: 161) by the authors on control subjects were used for comparing the tests.

The results showed that the two-dose is overly sensitive, disagreeing with the other tests and yielding diabetic results in non-diabetic subjects with significant frequency. The postprandial blood sugar correlates relatively well with the intravenous and standard tests. Due to its simplicity it is of value as a presumptive test. The intravenous test is less sensitive than the standard but both are satisfactory for clinical diagnostic use. Of the four tests evaluated in this study the standard oral test is both the most sensitive as well as the most specific. The twenty-four-hour urine glucose excretion did not appear to correlate with the degree of carbohydrate dysmetabolism, probably because of variations in renal threshold.

ANEMIA IN RENAL INSUFFICIENCY: STUDIES AFTER BILATERAL NEPHRECTOMY. *E. E. Muirhead, F. Jones (by invitation) and Arthur Grollman.* (From the Department of Internal Medicine, Southwestern School of the University of Texas, Dallas, Tex.)

Bilateral nephrectomy of the dog has been demonstrated to be associated with hypertension, vascular degeneration and anemia. The anemia has been studied in the rabbit. Bilateral nephrectomy of the rabbit, without removal of waste products, has been followed with survival of three to eight days. The red blood cell volume, whole blood volume and plasma volume have been measured by the T–1824 method and the P^{32} method. The red cell mass decreased during this time to levels of 30 to 50 per cent below the control. The whole blood volume decreased to a lesser extent and the plasma volume to a still lesser extent. The hematocrit was reduced. This anemia was associated with a slight increase in serum bilirubin, a significant increase in the stool urobilinogen, an increase in serum iron, an increase in the serum iron-binding capacity, a slight increase in the percentage saturation of the iron-binding globulin of serum and increase in the iron content of the spleen and liver. No occult blood was noted in the stool. The white blood count, platelets and reticulocytes were undisturbed. The bone marrow remained cellular. The blood non-protein nitrogen and urea were markedly elevated.

It is concluded that bilateral nephrectomy of the rabbit leads to a rapid anemia due mainly to a hemolytic mechanism. The nature of the hemolytic mechanism remains to be demonstrated.

* Expressed as equivalent of free drug.

EFFECTS OF ANEMIA ON HEPATIC BLOOD FLOW AND SPLANCHNIC METABOLISM. *J. D. Myers.* (From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

The hepatic blood flow (HBF) in a series of twenty human subjects with anemia, usually severe, is increased as is the cardiac output, only when the hemoglobin is below 7 gm. HBF then is usually proportional to the cardiac output. The hepatic arteriovenous oxygen difference is necessarily subnormal because of the low oxygen capacity of anemic blood. Splanchnic oxygen consumption (SP O_2) is moderately supernormal but in keeping with the elevated total oxygen consumption which anemic patients maintain. Because of the low oxygen-carrying power of anemic blood and the somewhat supernormal SP O_2 hepatic venous blood is considerably less saturated with oxygen than is normal (41 per cent as compared to 77 per cent). This anoxia may be important in the centrolobular hepatic necrosis which is often found in severe anemia.

The high oxygen extraction across the splanchnic circulation prevents any substantial increase in SP O_2 without a further rise in the already augmented HBF. Accordingly, in anemia SP O_2 is not significantly increased after parenteral amino-acids for oxidative deamination, in contrast to a normal 50 per cent increase. The elevated HBF in anemia is combined with a subnormal hepatic A-V glucose difference to provide a splanchnic glucose production within normal limits. Likewise, the elevated HBF and a low percentage extraction of bromsulfalein make for a normal total BSP removal by the liver.

MECHANISM OF VOLUME CHANGES AND MOBILIZATION OF INTERSTITIAL PROTEINS BY SALINE INFUSIONS. *H. S. Mayerson and (by invitation) Karlman Wasserman.* (From the Department of Physiology, Tulane University School of Medicine, New Orleans, La.)

Dogs, anesthetized with pentothal, are infused with 1,000 ml. of physiologic saline solution administered over a period of thirty minutes. Thirty minutes after such infusions in intact animals there is an increase in the amount of total circulating plasma albumin of up to 20 per cent. The plasma volume is increased but the rise is always considerably less than the amount of the infusion. Concomitantly, there is an increased volume flow of thoracic duct lymph and an increased rate of albumin return which is about six times that of the control period. When the lymph is not returned to the circulation or the lymph replaced by saline, the total circulating albumin actually decreases about 30 per cent in three hours. Likewise, there is a minimal increase or an actual decrease in plasma volume. Experiments with albumin labeled with radioactive iodine (I^{131}) indicate that there is no new albumin involved in the process. Our experiments suggest that the infusion results in the transudation of increased amounts of fluid and albumin from the circulation. This then dilutes the interstitial fluid containing a higher concentration of albumin than the transudate and results in lymph of a relatively high albumin concentration. This mobilization of interstitial albumin emphasizes the importance of lymph in the return of proteins to the circulation.

ALTERATION OF THE INITIAL INFLAMMATORY RESPONSE BY CORTISONE. *Max Michael, Jr. and C. Merrill Whorton (introduced by James V. Warren).* (From the Medical and Laboratory Services, Lawson Veterans Administration Hospital, and the Departments of Medicine and Pathology, Emory University School of Medicine, Atlanta, Ga.)

The present study was undertaken to determine the influence of cortisone on the early inflammatory reaction. Croton oil was used to induce inflammation in the skin of two groups of rabbits, one receiving cortisone and one serving as a control. The gross and microscopic lesions of both groups were compared at frequent intervals. Striking differences were noted in the gross appearance of the lesions in the two groups. In the control animals an early, intense erythema appeared and progressed to spreading necrosis within forty-eight hours. In contrast, in animals receiving cortisone only mild erythema appeared and no necrosis occurred. The early, acute inflammatory response, as manifested by capillary engorgement, margination and migration of leukocytes, and local edema was markedly inhibited by cortisone. Such changes were noted as early as thirty minutes after application of the irritant.

Previous studies have shown that cortisone inhibits the formation of granulation tissue. This is one of the explanations given for the spread of many bacterial infections under the influence of cortisone. The present study demonstrates the effect of cortisone on the initial inflammatory response and may shed additional

light on the adverse effects of this steroid on bacterial infections. The possible mechanisms of the action of cortisone on inflammation and the implications that can be drawn from these findings will be discussed.

EFFECTS OF OXYGEN THERAPY ON CEREBRAL FUNCTIONS IN PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA. *John L. Patterson, Jr., Albert Heyman and T. Whatley Duke (by invitation).* (From the Department of Medicine, Emory University School of Medicine, Atlanta, Ga.)

The administration of oxygen therapy to patients with chronic pulmonary disease occasionally produces undesirable mental and neurologic reactions.

The cerebral blood flow (CBF) and metabolism ($CMRO_2$) were studied before and during oxygen therapy in nine patients with chronic pulmonary emphysema and cor pulmonale and in eight control subjects. The mean CBF in these patients was 60 cc./100 gm. brain/min.—slightly greater than normal, while the $CMRO_2$ was 2.8 cc., a value slightly below that of the control subjects. In general, the patients with the lowest values for arterial oxygen tension and the highest values for blood carbon dioxide tension showed the highest blood flow values. During inhalation of 85 to 100 per cent concentration of oxygen the mean CBF value rose to 67 cc. while the $CMRO_2$ remained unchanged. Six of the nine patients showed a marked increase in CBF, with a maximum value of 113 cc. In contrast, the control subjects showed a slight fall in CBF.

The changes in cerebral function following oxygen therapy in these patients are caused by the effects of a depression in ventilation. The resultant elevation in arterial pCO_2, although moderate in degree, apparently exerted a greater effect on cerebral vessels than the marked rise in pO_2 and is believed to be responsible for elevation of CBF and some of the mental changes observed in such patients.

FLUCTUATIONS IN CRANIAL ARTERIAL TONE AS MEASURED BY HISTAMINE "RESPONSIVENESS." *J. B. Pfeiffer, Jr. and E. C. Kunkle (introduced by Eugene A. Stead, Jr.).* (From the Department of Medicine (Neurology) of the Duke University Hospital and School of Medicine, Durham, N. C.)

The contractile state of cranial arteries has, by inference, been tied to the mechanism of vascular headache, such as that of migraine or febrile states. On the assumption that headache might be more easily induced by histamine (a drug known to dilate intracranial arteries) when cranial arterial tone is low, the present investigation was planned to determine: (1) whether day-to-day variations in the tone of the cranial arterial tree are detected by the "responsiveness" of the subject to histamine and (2) whether such fluctuations can be correlated with other biologic variables, such as mood, energy and tension.

Histamine phosphate was given to five adult subjects by daily intravenous injection for fourteen to eighteen days in an amount found by preliminary trial to produce mild headache. Prior to each injection notes were made of the subject's activities, attitudes and feeling state. Mild or moderate day-to-day fluctuations in the intensity of histamine-induced headache were noted in all subjects. It was observed that resistance to histamine-induced headache was increased under conditions which promoted relaxation and feelings of accomplishment and was diminished with fatigue and feelings of discouragement. In three of the subjects the general trend during the experimental period was one of decreasing headache from successive injections (possibly representing "desensitization") but in all but one subject the intradermal response to histamine was as large at the end of the injection series as at the beginning.

SERUM AND URINARY PROTEINS IN MULTIPLE MYELOMA. *R. W. Rundles, G. R. Cooper (by invitation), and R. W. Willett (by invitation).* (From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

The overgrowth of plasma cells in multiple myeloma is generally associated with abnormal protein components in the serum and often with Bence Jones proteinuria. The significance of the serum increments in this disease and the relationship of the serum to the urinary proteins has been obscure.

The abnormal proteins in thirty patients with multiple myeloma have been studied by electrophoresis, diffusion and ultracentrifuge measurements. The major findings were as follows: (1) The amount of abnormal protein in the serum showed no constant relation from patient to patient to the amount of Bence Jones protein excreted in the urine. (2) The electrophoretic mobility of urinary Bence Jones protein in a given patient was the same, faster or much

faster than that of the abnormal serum increment. (3) Diffusion and sedimentation measurements in nine patients showed that the abnormal serum components were of high molecular weight, 120,000 to 200,000. The molecular weight of the Bence Jones proteins excreted by nine patients varied from 24,000 to 90,000. (4) Two patients with extensive disease had no proteinuria and normal serum electrophoretic patterns.

Our observations are consistent with the hypothesis that Bence Jones proteins are moieties of low molecular weight, filterable through the glomeruli, which are derived from the abnormal serum components.

INFLUENCE OF GASTRIC FUNCTION ON THE DURATION OF ANTACID ACTION. *Arthur M. Schoen (introduced by J. Murray Kinsman)*. (From the University of Louisville Institute for Medical Research, Louisville, Ky.)

Antacids, when administered hourly in the usual clinical dosage and alternated with milk and cream, do not maintain constant adequate neutralization of gastric contents. For example, when the antacid was administered as 2 gm. doses (15 cc.) of U. S. P. magnesium trisilicate, prepared in an emulsion, gastric acidity ranged between 1.48 and 2.05 thirty minutes following the antacid. When the antacid dosage was increased to 4 gm. hourly, the pH was maintained between 5 and 6 at thirty minutes. When $\frac{1}{75}$ gr. of atropine sulfate was administered orally every two hours along with the hourly administration of 2 gm. doses of magnesium trisilicate, the pH then also ranged between 5 and 6 thirty minutes after the antacid.

It is the purpose of this report to show (1) results of quantitative studies on the effect of atropine sulfate on diurnal and nocturnal secretion and emptying in nine persons with peptic ulcer studied by two methods, (2) that the duration of intragastric neutralization by an antacid is determined principally by the rate of gastric emptying rather than by the rate of gastric secretion, and (3) that the relationship between the rate of secretion, rate of emptying and duration of antacid action can be expressed by the formula

$$s\left[\log\left(\frac{75 + (s - e)t}{75}\right)\right] = (e - s)\log 0.154,$$

where s = rate of gastric secretion, e = rate of gastric emptying and t = duration of antacid action.

RESPONSE OF THE HEART TO VAGAL STIMULATION IN HYPERCAPNIA AND HYPOXIA. *W. C. Sealy, Jerome Harris, Arnold Botwin (by invitation) and W. G. Young, Jr. (by invitation)*. (From the Depts. of Medicine and Surgery, Duke University School of Medicine, Durham, N. C.)

Beecher has called attention to the prevalence of hypercapnia in open chest operations. Our own observations suggested that both vagal stimulation and hypercapnia played a role in cardiac arrest during the Blalock-Taussig procedure. To investigate this possibility the right or left cervical vagus was stimulated in dogs until vagal escape of the heart occurred. The response to stimulation was measured by the duration of electrocardiographic asystole. It was found that the duration of asystole varied directly with the percentage of carbon dioxide in the inspired air. Hypoxia shortened the duration of asystole, particularly after it had been prolonged by hypercapnia. Neither mild hypercapnia nor hypoxia alone caused serious electrocardiographic abnormalities but the combination led to the well known asphyxial alterations. Although cardiac arrest was not produced by vagal stimulation alone, it is suggested that the enhanced vagal effect in hypercapnia may be an important factor in a decreased cardiac tolerance to hypoxia and eventual cardiac arrest.

EFFECT OF L-NOR-EPINEPHRINE ON THE CEREBRAL CIRCULATION IN NORMOTENSIVE MALES. *Willis Sensenbach, Leonard Madison and Lamar Ochs (introduced by Arthur Grollman)*. (From the Department of Medicine, Southwestern Medical College of the University of Texas, and the Department of Medicine, Veterans Administration Hospital, Dallas, Tex.)

These studies were made to determine the effect of nor-epinephrine on the cerebral circulation. The cerebral blood flow was measured before and during the height of the pressor response induced by the intramuscular administration of L-nor-epinephrine in oil to fifteen young normotensive males. The changes in the cerebral circulation and arterial and cerebral venous blood gases are shown in the table which follows.

The results indicate that: (1) L-nor-epinephrine is a powerful cerebral vasoconstrictor. (2) The increase in arterial blood pressure (31 per cent) induced by L-nor-epinephrine is not of sufficient magnitude to compensate for the larger increase in cerebral vascular resistance (58 per cent); the cerebral blood flow is therefore reduced

(20 per cent). (3) Oxygen consumption of the brain remains essentially unchanged since the reduction in blood flow is associated with an increase in A-VO_2.

	Control Flow Mean	Experimental Flow Mean	Per cent Change	P Value
Femoral arterial blood pressure mm. Hg...	74	97	+31	.01
Cerebral blood flow cc./min./100 gm...	69	55	−20	.1
Cerebral vascular resistance mm. Hg, co./min./100 gm...	1.2	1.9	+58	.01
Cerebral oxygen consumption cc./min./100 gm...	4.30	4.07	−5	.5
Arterial CO_2 content, vol. %.....	47.50	45.20	−5	.01
Arteriocerebral venous oxygen difference, vol. %.....	6.40	7.70	+20	.01

STUDIES ON PRESSURES IN AN OCCLUDED HEPATIC VENULE AND THEIR POSSIBLE RELATIONSHIP TO PORTAL VENOUS PRESSURE. *W. J. Taylor (by invitation) and J. D. Myers.* (From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

No reliable clinical measurement of portal venous pressure, short of laparotomy and direct observation, has been possible in man. The demonstration that "pulmonary capillary" pressure corresponds to left atrial pressure suggested a similar exploration of pressure in the more complex hepatic circulation.

Measurements in cats (in which the ratio of portal venous blood to hepatic arterial blood supplying the liver is most like man) of pressures recorded through a venous catheter passed occlusively into a distal hepatic venule gave a mean value of 9.5 mm.Hg. Almost simultaneous direct measurements of portal venous pressure were in close agreement with a mean difference of −1 mm.Hg.

In preliminary studies on patients with clinical manifestations of portal hypertension pressures measured through an open end venous catheter in an occluded hepatic venule were two- to fourfold higher than similar measurements in normal subjects. These pressures in the portal hypertensives agreed in magnitude with portal venous pressures recorded by others at laparotomy. An arterial pulse wave has also been recorded from the hepatic venule in both cirrhotic and normal subjects. This may suggest that in certain circumstances there is open communication between the arterial and hepatic venous circulation in the liver.

CELLULAR CHANGES IN THE ANTERIOR PITUITARY OF DOGS RENDERED HYPERTENSIVE BY CONSTRICTION OF THE RENAL ARTERIES OR BY BILATERAL NEPHRECTOMY. *E. H. Voigt (by invitation), J. C. Meadows (by invitation), W. D. Davis, Jr. and Thomas Findley.* (From the Departments of Anatomy and Medicine, Tulane University School of Medicine, and the Department of Internal Medicine, Ochsner Clinic, New Orleans, La.)

Differential cell counts on the anterior pituitaries of four adult normal mongrel dogs were:

Sex	Acidophiles (per cent)	Basophiles (per cent)	Chromophobes (per cent)
M	39.4	11.2	49.4
M	38.8	6.3	54.9
F	39.5	3.6	56.9
F	42.1	14.1	43.8
Average	40	9	51

In one dog with a mean arterial blood pressure (direct femoral artery puncture) varying from 150 to 205 mm./Hg for six months following bilateral application of Goldblatt clamps to the renal arteries the count was as follows: acidophiles 53.4 per cent, basophiles 2.1 per cent, chromophobes 44.5 per cent. In another dog whose blood pressure reached 170 mm./Hg one month after unilateral nephrectomy and clamping of the opposite renal artery the proportions were: 72.2 per cent, 2.7 per cent and 25.1 per cent, respectively.

Through the courtesy of Dr. Arthur Grollman the pituitaries of five dogs rendered hypertensive by bilateral nephrectomy were compared.

Experimental renal hypertension appears to be accompanied with a relative increase in the number of anterior pituitary acidophiles and a relative decrease in the numbers of basophiles and chromophobes. The basophiles not infrequently assumed the appearance of Crooke cells. Since the changes are produced by "renal

ischemia" and bilateral nephrectomy, it is possible that the renal tubules normally secrete a substance which inhibits the function of the pituitary acidophiles.

Dog	Days of Survival	Final BP	Acidophiles (percent)	Basophiles (percent)	Chromophobes (per cent)
14	70	160	68.4	1.4	30.2
16	40	200	47.3	3.9	48.8
18	20	160	51.3	6.4	42.3
23	30	180	73.2	2.4	24.4
30	39	170	47.7	8.1	44.2
		Average	57.6	4.4	38

STUDIES ON A PATIENT WITH A PHEOCHROMOCYTOMA. *William P. Walsh, Laurence H. Kyle and Donald C. Edgren (by invitation) (introduced by Harold Jeghers).* (From the Department of Medicine, Georgetown University School of Medicine and the Georgetown University Hospital, Washington, D. C.)

A twenty-nine year old woman with paroxysmal hypertension, attacks of headache, vomiting and faintness, and a history of precordial pain with unconsciousness diagnosed as a heart attack was studied before and after the removal of a pheochromocytoma. A marked hypertensive response followed the injection of histamine. A similar rise was induced by the administration of tetraethyl ammonium; and when the histamine test was repeated six hours later, no hypertensive response occurred. Histamine-induced hypertension was only moderately inhibited by benzodioxane (17 mg.) but a more marked suppression of the pressor response was effected by the use of priscoline (12.5 mg.). The BMR was plus 34 per cent, there was neither hyperglycemia nor glycosuria, urinary 17-ketosteroid excretion was 5.5 mg. in twenty-four hours and there was no depression of circulating eosinophils. On the supposition that an increased amount of circulating epinephrine might cause insensitivity to exogenous adrenalin, an epinephrine-eosinophil test was performed. Subcutaneous injection of 0.4 mg. resulted in a slight rise in eosinophils and caused no symptoms. After removal of the tumor the test was repeated in the same fashion. The eosinophils decreased from 140/mm.^{3} to 40/mm.^{3} and the patient experienced mild symptoms of epinephrine stimulation. By means of paper chromatography the tumor was shown to contain about 90 per cent nor-epinephrine and 10 per cent epinephrine.

LOCAL PERIPHERAL RESISTANCE IN CANINE HYPERTENSION. *Arnold H. Williams and Henry A. Schroeder.* (From the Hypertension Division, Department of Internal Medicine and the Oscar Johnson Institute, Washington University School of Medicine, St. Louis, Mo.)

The asystolic arterial pressure gradient, the descending curve of pressure after arterial occlusion, is a direct measure of local arteriolar run-off and indicates the degree of vasoconstriction in a peripheral vascular bed. The pressure six seconds after occlusion (EP_6) is the best approximation of conditions found. Renal, mesenteric, brachial and femoral resistance was measured in anesthetized dogs with acute and chronic neurogenic and chronic renal hypertension, and compared to normal values. The mesenteric area contributed the largest part of the increase in peripheral resistance in all types of hypertension, and EP_6 was correlated with diastolic pressure. Renal resistance bore no relation to the existence or extent of hypertension; in renal hypertension, however, marked vasoconstriction distal to Goldblatt clamps was a constant finding. The femoral and brachial areas were only slightly constricted in all types except the acute neurogenic in which they were markedly constricted. Calculations based on pressure flow curves found for these areas gave rough indices of the relative degree of vascular tonus or size in each bed.

CLINICO-PATHOLOGIC EXAMPLES OF UNTOWARD EFFECTS OF CORTISONE IN PATIENTS WITH BACTERIAL INFECTIONS. *C. Merrill Whorton, Max Michael, Jr., Martin M. Cummings and W. L. Bloom (introduced by Paul B. Beeson).* (From the Laboratory and Medical Services, Lawson Veterans Administration Hospital and Departments of Pathology and Medicine, Emory University School of Medicine, Atlanta, Ga.)

Recent observations have indicated that cortisone may exert a harmful effect on many patients with bacterial infections. Although the mechanism of this is not clearly understood, several possibilities present themselves. Among these are the ability of this substance to suppress the early inflammatory response, to suppress formation of granulation tissue, to lower natural resistance and to mask the usual signs of infec-

tion. This report is concerned with a group of patients who received cortisone for a variety of diseases and who either developed infections while under therapy or who had a rapid spread of pre-existing, otherwise benign, lesions while receiving the hormone. Included in this series are two patients who showed extensive tuberculosis at autopsy although little evidence for it existed during life; patients with spreading superficial skin infections that usually are well localized; and one patient with pneumococcal pneumonia discovered at postmortem examination but unrecognized during life. Histologic studies will be presented in an effort to explain these effects. The enhancement of bacterial infections can be a treacherous side effect of cortisone and must be constantly kept in mind while this substance is being administered.

TRIETHYLENE MELAMINE THERAPY IN LEUKEMIA AND LYMPHOMATOUS DISEASE. *Robert W. Willett (by invitation), Charles L. Rast (by invitation), John C. Muller (by invitation) and R. W. Rundles.* (From the Department of Medicine, Duke University School of Medicine, Durham, N. C.)

Forty-eight patients with hematologic disorders and malignancies have been treated with triethylene melamine in a period of five months. Diagnoses and results are tabulated.

Diagnosis	No. Treated	Effect on Disease		
		Beneficial	None	Inconclusive
Hodgkin's disease	12	8	0	4
Lymphoma, lymphocytic	2	2	0	0
Lymphoma, lymphoblastic	8	4	3	1
Lymphoma, giant follicular	3	1	0	2
Lymphoma, reticulum cell	1	1	0	0
Leukemia, chronic lymphocytic	8	6	0	2
Leukemia, subleukemic lymphocytic	2	0	0	2
Leukemia, chronic myelogenous	2	0	0	2
Leukemia, acute myeloblastic	1	0	0	1
Leukemia, acute myelomocytic	1	0	0	1
Multiple myeloma	2	0	2	0
Polycythemia vera	2	0	0	2
Lymphoepithelioma	3	3	0	0
Teratoma testis	1	0	1	0

Side effects of the drug after oral administration include anorexia, nausea, vomiting, abdominal and pleuritic pain. Adequate amounts of the chemical can be given to most patients without producing significant discomfort. The chemical can be given in doses of 2.5 to 5 mg. every one to two days. Effective doses range from 10 to 40 mg. and must be individualized. Maximal depression of cellular growth, including cells from the bone marrow, occur in seven to ten days. The general effect of the chemical parallels that of nitrogen mustard compounds and is of definite promise in treating proliferative diseases of the lymphoid tissues.

USE OF DEXTRAN IN THE TREATMENT OF SHOCK. *Joseph S. Wilson (by invitation), Joseph T. Doyle (by invitation), E. Harvey Estes, Jr. (by invitation) and James V. Warren.* (From the Departments of Medicine and Physiology, Emory University School of Medicine and Grady Memorial Hospital, Atlanta, Ga.)

The high molecular weight of dextran, a glucose polymer, indicates that it may be clinically useful in expanding plasma volume. Circulatory changes accompanying the intravenous administration of dextran have been studied in normal subjects following phlebotomy and in patients in shock.

In five normal subjects a phlebotomy of 450 to 900 cc. resulted in a fall in pulmonary arterial pressure commensurate with the reduced blood volume. The cardiac output showed no change or a slight fall. Following dextran there was an expansion of the general blood volume approximately to control levels and an average fall in hematocrit reading of 14 per cent. The hematocrit reading was depressed throughout the observation period indicating that the dextran remained intravascular. In every instance the pulmonary arterial pressure returned to control levels and the cardiac output rose.

Fifteen patients in shock have received dextran with uniformly satisfactory results. In twelve cases the shock was due to blood loss, in two cases to extensive trauma and in one to diabetic acidosis. In all patients there was evidence of circulatory improvement. In two instances, mild thermal reactions occurred, the cause of which remained obscure.

These results indicate that dextran is an effective plasma substitute in the therapy of shock.

RESPONSE OF MEGALOBLASTIC ANEMIA OF INFANCY TO CITROVORUM FACTOR. *Calvin W. Woodruff (introduced by William J. Darby).* (From the Department of Pediatrics and Division of Nutrition of the Departments of Medicine and Biochemistry, Vanderbilt University School of Medicine, Nashville, Tenn.)

Two infants with megaloblastic anemia of infancy were treated with 500,000 units of citrovorum factor. A clinical and hematologic

response followed which was comparable to that seen following treatment with 200 micrograms of folic acid. The relative effectiveness of citrovorum factor and folic acid in this type of anemia will be discussed.

INFECTIONS DUE TO PSEUDOMONAS AERUGINOSA DEVELOPING DURING ANTIBIOTIC THERAPY. *Ellard M. Yow (introduced by James A. Greene).* (From the Department of Medicine, Baylor University College of Medicine, Houston, Tex.)

Recently developed antimicrobial agents are capable of controlling almost all of the bacterial infections. Pseudomonas aeruginosa, however, is outstanding in its resistance to these agents. Infections due to this organism have consequently increased in their relative importance and, we believe, in their actual frequency. This paper is concerned with observations made in eighteen patients with pseudomonas infections. The infections have been classified into those which were considered primary and unrelated to previous drug therapy, those due to the failure of prophylactic antibiotic therapy when pseudomonas was introduced through instruments or solutions, and those resulting from the removal of other bacteria from diseased tissue or from patients in whom the immune mechanisms were not adequate. The problem of therapy of pseudomonas infection is discussed.

Case Reports

Thyrotoxicosis Factitia*

Measurement of the Thyroid Gland I^{131} Uptake and Serum Protein-bound I^{131}

DAVID L. CHAMOVITZ, M.D., MARVIN H. SLEISENGER, M.D. *and* A. STONE FREEDBERG, M.D.
Boston, Massachusetts

THE diagnosis of thyrotoxicosis factitia (also called alimentary or exogenous thyrotoxicosis), the morbid condition caused by the surreptitious ingestion of thyroid, presents a challenge to the physician. In a patient with the symptoms and signs of thyrotoxicosis there may be little to make one suspect the diagnosis of thyrotoxicosis factitia. The absence of an enlarged thyroid gland or exophthalmos may be suggestive of thyrotoxicosis factitia[1] but is not inconsistent with endogenous thyrotoxicosis.[2] Contrariwise, exophthalmos[3–7] and an enlarged thyroid gland[3] have been observed during a period of thyroid administration.

The usefulness of tracer studies with I^{131} to determine thyroid function has been established.[8–11] The special significance of these studies in the differential diagnosis of endogenous and exogenous thyrotoxicosis has been reported by Skanse and Riggs.[7] In two cases of thyrotoxicosis factitia these authors demonstrated almost complete excretion of a tracer dose of I^{131} into the urine in forty-eight hours; external screening of the patients' bodies with a Geiger-Mueller tube revealed no remaining radioactivity.

This paper presents the case study of a patient with thyrotoxicosis factitia. The history, physical examination and laboratory findings including basal metabolic rate, serum cholesterol, circulation time, serum protein-bound iodine and, especially, the urinary excretion of a tracer dose of I^{131} were consistent with the diagnosis of endogenous thyrotoxicosis; the low uptake of an I^{131} tracer dose by the thyroid gland as measured by external counting led to the correct diagnosis of thyrotoxicosis factitia. The finding of a normal concentration of serum protein-bound I^{131} twenty-four hours after the administration of the tracer dose will also be described and its value in the diagnosis discussed.

METHODS

A low iodine diet was given the patient throughout the period of study. Tracer doses of I^{131} carrier-free ranging from 122 to 195 microcuries (Fig. 1, day of administration indicated by arrow) were administered orally to the patient in a fasting state. The I^{131} uptake by the thyroid gland was measured by the four Geiger-Mueller tube method of direct counting[12–13] developed in these laboratories. The I^{131} urinary excretion was also measured by this method.[14]

Measurement of the serum protein-bound I^{131} and total serum I^{131} was made by a modification[15] of the method previously reported.[16] Increased accuracy has been accomplished by measuring total volumes of 8 cc. in stainless steel cups (surface area, 32 sq. cm.) with a beta-ray counting tube; approximately 1.5×10^{-5} microcuries I^{131} per cc. of serum gives a counting rate equalling two times background.

* From the Medical Research Department, Yamins Research Laboratory, Beth Israel Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass. This work was carried out under a contract between the Office of Naval Research, the Atomic Energy Commission and the President and Fellows of Harvard College.

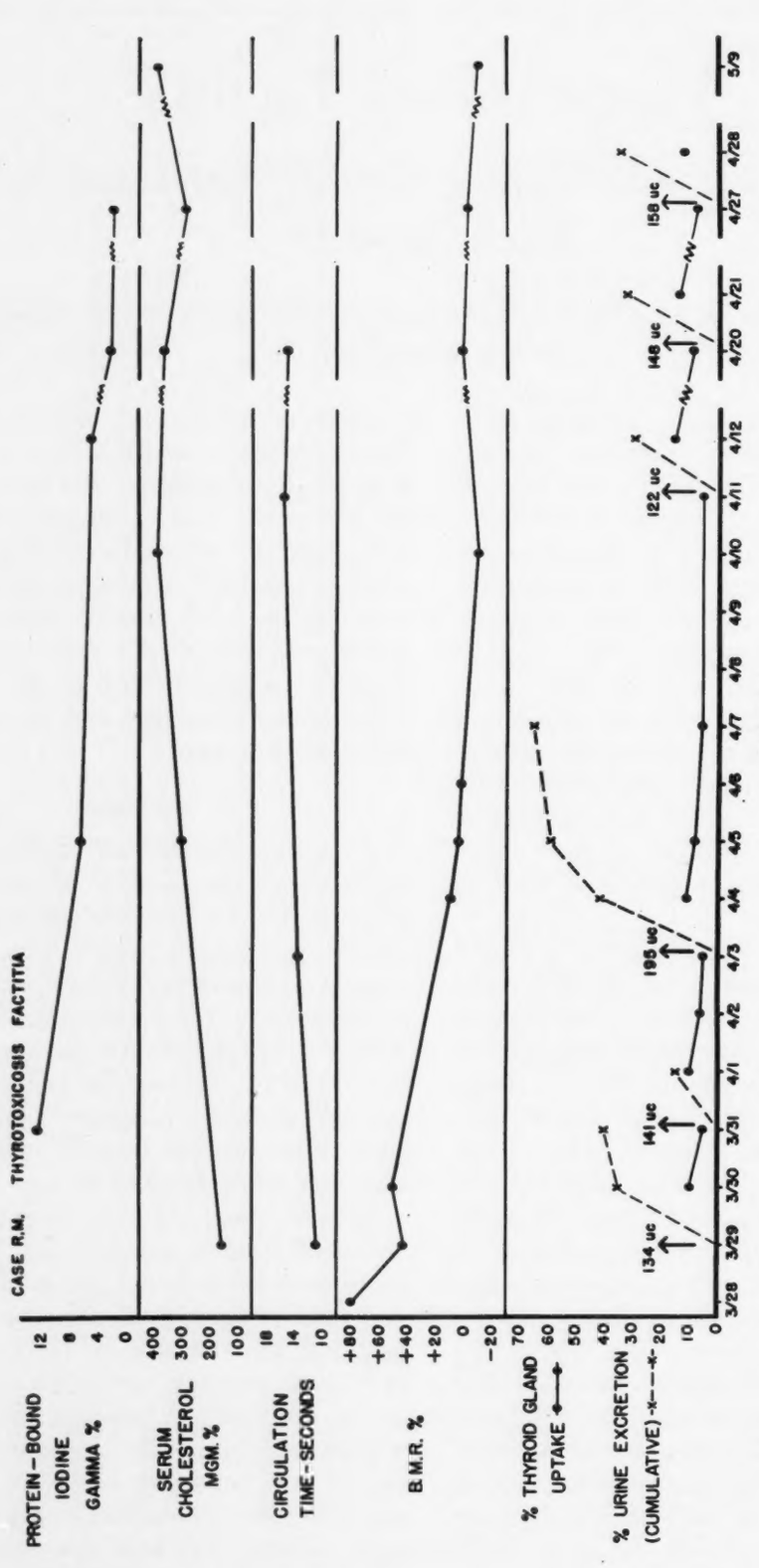

FIG. 1. The laboratory findings in a patient with thyrotoxicosis factitia. No thyroid was ingested after March 28, 1950. Tracer doses, marked by the arrows, were administered on March 29 and 31, April 3, 11, 20 and 27. During the period March 28 to April 12 the patient was hospitalized. The remaining studies were carried out on ambulatory basis. Intervals between periods of study are represented by the broken line curves.

The serum protein-bound iodine measurements* were carried out in duplicate by a modification of the method described by Barker.[17]

CASE REPORT

R. M., B. I. H. No. M11073, a thirty-five year old white married woman, entered the hospital on March 27, 1950, because of palpitations. She had been well until three months before admission when she experienced dyspnea on exertion, fatigability and extreme irritability with frequent crying spells. Simultaneously, her appetite decreased and she began to lose weight. Two months before admission she noticed heat intolerance and night sweats. She also experienced one episode of syncope. A physician advised bed rest and administered barbiturates, bromides and quinidine. Six weeks before entry she noted palpitation, a rapid pulse rate and increasing dyspnea on slight exertion. Digitalis was administered without benefit. Intermittent bouts of diarrhea, marked tremor and menorrhagia had been noted. Because of continued weight loss (20 pounds), palpitation, rapid pulse and weakness she entered the hospital. The patient had not taken iodine containing compounds or other antithyroid drugs. She denied ingestion of thyroid medication.

Several paternal relatives had had surgery for goiter and had had diabetes mellitus.

Her home environment constituted a major problem. She lived with her husband, two children, mother, father and sister in a small apartment. Her mother was invalided by severe arthritis and her father was not well, working only part time as a clothes presser. The sister was incapacitated by rheumatic heart disease with congestive failure. Her two children were ill and required attention; a seventeen year old son had epilepsy and a thirteen-year old daughter had numerous allergic manifestations and recurrent fever. The patient's husband, aged thirty-seven, had not worked gainfully during the previous five years because of recurrent painful purpura. The patient disclosed the information that her husband's purpura was due to self-inflicted trauma. The family was supported by charitable agencies.

Physical examination revealed that the patient was a poorly nourished, haggard, anxious woman appearing older than her stated age. Her temperature was 99.6°F., pulse rate 110, respirations 28 and blood pressure 170/70. Her skin was moist, warm and flushed. Her eyes were bright with a staring expression; there was minimal bilateral lid lag and slightly impaired convergence but no exophthalmos. The thyroid was palpable but not enlarged; a systolic bruit was audible over it. The heart was normal in size and shape; the rhythm was regular with occasional premature contractions. The pulmonary second sound was accentuated. No cardiac murmurs were heard. The lungs were clear to percussion and auscultation. The peripheral pulses were bounding and marked capillary pulsations were noted. There was a fine tremor of the extended hands and frequent choreiform movements of the arms and legs. There was minimal pitting edema of the ankles.

Laboratory data showed that four urine examinations were negative except for green reduction of Benedict's reagent. The hemoglobin was 12.0 gm. per 100 cc., the red blood cell count 4.0 million per cu. mm. The white blood cell count was 6,900 with a normal differential count. Two stool examinations for occult blood were negative. The blood non-protein nitrogen was 23 mg. per 100 cc. The fasting blood sugar was 82 mg. per 100 cc. One and two and one-half hours after the oral administration of 100 gm. of glucose the blood sugar was 266 mg. per 100 cc. and 248 mg. per 100 cc., respectively. The serum cholesterol was 157 mg. per 100 cc. The blood Hinton test was negative. The basal metabolic rate was +80 per cent. The arm to tongue circulation time (decholin) was eleven seconds. A chest roentgenogram was normal; there was no evidence of substernal goiter. The electrocardiogram revealed sino-auricular tachycardia (rate 105) and T wave changes consistent with digitalis effect.

The clinical course was as follows: The admission diagnosis was thyrotoxicosis. On the third hospital day on a regimen of bed rest and mild sedation the patient was somewhat improved with diminished perspiration and slowing of the sleeping pulse rate to 80. The subsequent laboratory data are illustrated in Figure 1. An I^{131} tracer study on the third hospital day showed 9 per cent thyroid gland uptake in twenty-four hours. Screening of the body, especially over the abdomen, with a Geiger-Mueller tube revealed no localization of I^{131} beyond the thyroid area. The low I^{131} uptake was inconsistent with hyperthyroidism and the diagnosis of thyrotoxi-

* The authors wish to thank Mrs. Dorothy Pratt and Dr. John Stanbury for these determinations.

cosis factitia was thought likely. The patient's property was searched and enteric-coated 30 to 60 mg. thyroid tablets were found. She admitted that during the three months before hospitalization she had taken about 100 to 200 of these thyroid tablets per week in an effort to commit suicide. The thyroid had been prescribed for her son several years previously and she had repeatedly renewed the prescription. She had ingested no thyroid after the second hospital day.

Therapy was directed toward adjusting the patient's difficult home situation. She had many interviews with staff psychiatrists. On the sixth hospital day the tremor was gone and the patient was eating and sleeping well. On the ninth hospital day the basal metabolic rate was +10 per cent; arm to tongue circulation time (decholin) was 13.5 seconds and the serum cholesterol was 301 mg. per 100 cc. On the sixteenth hospital day the serum cholesterol was 394 mg. per 100 cc.; the basal metabolic rate was −13 per cent and the circulation time was 15.5 seconds. She was discharged on the seventeenth hospital day feeling well.

During the ensuing month signs and symptoms suggestive of mild hypothyroidism developed in the patient. She noted decreased perspiration, numbness of fingers and toes and arthralgias. The cholesterol remained elevated, 384 mg. per 100 cc., the basal metabolic rate, slightly depressed, between −6 and −15 per cent. The twenty-four-hour thyroid gland I^{131} uptake was unchanged, 12 per cent (Fig. 1, 158 uc. I^{131} administered April 27th).*

COMMENTS

On the basis of the history, physical examination and initial laboratory tests the clinical diagnosis in this patient was moderately severe thyrotoxicosis. The following evidence was consistent with this diagnosis: nervousness, irritability, heat intolerance, increased perspiration, palpitation, dyspnea on exertion, diarrhea, fatigability and a weight loss of 20 pounds in three months. She was poorly nourished and had moist, warm, flushed skin, tremulous hands and staring eyes with bilateral lid lag. Tachycardia was present. There was no exophthalmos or thyroid gland enlargement. The basal metabolic rate was elevated, the serum cholesterol diminished and the circulation time decreased. The serum protein-bound iodine of 12.4 gamma per cent (Fig. 1, upper curve) was confirmatory of the diagnosis of endogenous thyrotoxicosis. However, the twenty-four-hour thyroid gland I^{131} uptake of only 9 per cent was clearly inconsistent with this diagnosis. Further evidence against the diagnosis of endogenous thyrotoxicosis in this patient was provided by measurement of the protein-bound I^{131} of serum.[16,18,19] This measurement was made in this patient twenty-four hours after the administration of each tracer dose. The concentration of protein-bound I^{131} per cc. of serum per 150 microcuries of administered I^{131} varied between 14×10^{-5} and 27×10^{-5} microcuries, these values being in the euthyroid range.[16] The ratio of the measurement of the serum protein-bound I^{131} to the total I^{131} concentration was similarly normal[15] ranging between 9 and 13 per cent. Admission by the patient of thyroid ingestion confirmed the diagnosis of thyrotoxicosis factitia.

Previous observations[9,15,20] have shown that the administration of thyroid blocks the uptake of I^{131} by the thyroid gland. This blocking effect has been demonstrated by us in a patient with a nodular goiter and ovarian dwarfism. At the end of a seven-month course of thyroid administration (60 to 180 mg. daily) the twenty-four-hour thyroid gland uptake was 3 per cent of a tracer dose. Eight and twenty weeks following discontinuation of therapy with thyroid her thyroid gland uptake was 34 and 48 per cent, respectively. Greer[20] noted that 60 mg. of thyroid administered daily for one week was sufficient to inhibit the thyroid gland uptake of I^{131} in one-third of the subjects studied. Similar inhibition was obtained with 120 to 540 mg. of thyroid per day in the remaining subjects and persisted two to eleven weeks following cessation of thyroid administration. In the patient

* On January 23, 1951, approximately ten months after omission of thyroid extract the following normal findings were obtained: twenty-four-hour thyroid gland uptake, 33 per cent; basal metabolic rate, −7 per cent; and cholesterol 330 mg. per cent. The protein-bound iodine was 3.2 gamma per cent.

studied here the reduced uptake persisted for the four-week period of study following cessation of thyroid administration.

Farquharson[21] and Riggs[22] have demonstrated laboratory and clinical evidence of transitory hypothyroidism following the termination of a course of thyroid administration in euthyroid subjects, suggesting temporary arrest of thyroid gland activity by the exogenous thyroid. The clinical and laboratory findings, particularly the changes in serum protein-bound iodine, in the patient studied by us are consistent with this interpretation. From an initial value of 12.4 gamma per cent obtained two days following cessation of thyroid ingestion the serum protein-bound iodine fell in five days to 6.3 gamma per cent and in another fifteen days to 2.0 gamma per cent. (Fig. 1.) These values agree closely with those found by Riggs[22] who studied the effect of the ingestion of thyroid on the protein-bound iodine in euthyroid subjects. He noted that a daily dose of 0.6 gm. or more was necessary to elevate the protein-bound iodine above the range of normal. Within one week following the cessation of thyroid administration the protein-bound iodine fell to normal or subnormal limits; within another two to three weeks it was below normal limits. In the present case the serum protein-bound iodine four weeks after cessation of thyroid ingestion was still below normal. Riggs[22] noted a return to normal five weeks after cessation of thyroid.

Perkin[23] and Hurxthal[1] suggested that the quantitative determination of stable iodine in the urine would differentiate between endogenous and exogenous thyrotoxicosis. In spontaneous hyperthyroidism the twenty-four-hour urinary output of iodine is approximately 0.250 mg. The amount of iodine (0.2 per cent) present in 60 mg. of desiccated thyroid (U.S.P.) is equal to that amount excreted in a twenty-four-hour period in the urine by the normal individual, 0.110 to 0.150 mg. Accordingly, the urinary iodine increases as the amount of ingested thyroid increases.[23] A significant increase in urinary iodine excretion over that observed in the patient with endogenous thyrotoxicosis would be present in patients taking 0.3 or more gm. of thyroid per day. The patient observed by us had ingested 0.5 to 1.5 gm. daily. However, urinary excretion of stable iodine was not measured in our patient.

As exemplified by this patient in whom the urinary I^{131} excretion was consistent with the diagnosis of endogenous thyrotoxicosis, it is not an infrequent experience in our laboratory to find the determination of the urinary I^{131} excretion misleading (Fig. 1, bottom curve x------x). Not only is this the case in uncooperative patients such as this one, who evidently discarded specimens of urine, but also it must be emphasized that urinary I^{131} excretions in the thyrotoxic range have been observed in euthyroid patients with congestive heart failure or edema from other causes.[15] Contrariwise, urinary I^{131} excretions in the euthyroid range have been noted in endogenous thyrotoxicosis. This difficulty with urinary I^{131} excretion measurements is illustrated in the study by Skanse and Riggs of two cases of thyrotoxicosis factitia in whom the ingestion of thyroid was confirmed.[7,24] The first patient excreted 94.4 per cent of the tracer dose in twenty-four hours. The second patient, however, excreted only 53.2 per cent in this period. The absence of palpable thyroid tissue prompted screening of this patient with a Geiger-Mueller tube. Since no localization of I^{131} anywhere in the body (as in the first case) was found, a repeat tracer was administered, this time with the patient under close surveillance. The twenty-four-hour urinary I^{131} excretion was 85.7 per cent.

The measurement by external counting of the uptake of I^{131} by the thyroid gland and the measurement of serum protein-bound I^{131} offer more direct information concerning the activity of the thyroid gland than does measurement of the urinary I^{131} or stable iodine excretion. However, normal findings in the I^{131} studies may be obtained in thyrotoxic patients who have ingested, or have been given parenterally, stable

iodide (potassium iodide, diodrast and lipiodol) in one form or another. We have, however, not observed a blocked uptake to be so complete and to last so long as in this patient. It is likewise possible to produce a low thyroid gland I^{131} uptake and a high urinary excretion with the simultaneous administration of either 2-mercaptoimidazole or potassium thiocyanate with the I^{131} tracer in thyrotoxic patients.[15] No evidence was obtained that any of these drugs had been ingested by the patient.

SUMMARY

A study of a patient with thyrotoxicosis factitia is presented. The history, physical examination and laboratory findings, including basal metabolic rate, serum cholesterol, circulation time, serum protein-bound iodine and urinary excretion measurements of a tracer dose of I^{131} were consistent with the diagnosis of endogenous thyrotoxicosis. The low thyroid gland uptake of an I^{131} tracer dose, as measured by external counting, led, however, to the correct diagnosis of thyrotoxicosis factitia. The finding of a normal concentration of serum protein-bound I^{131} twenty-four hours after the administration of tracer doses is described and its value in the diagnosis discussed.

The clinical course and laboratory findings subsequent to cessation of thyroid ingestion indicating hypothyroidism are presented.

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Marfan's Syndrome (Arachnodactyly)*

GEORGE J. HAMWI, M.D.
Columbus, Ohio

ARACHNODACTYLY is a syndrome characterized by continued growth of the long bones with apparent lack of development of muscle and subcutaneous tissues. This primary anomaly is frequently associated with ocular and cardiovascular malformations.

In 1896 Marfan[1] first noted the skeletal characteristics of this syndrome in a five and one-half year old girl. The possibility that it was related to gigantism and acromegaly was mentioned. In 1902 Mery saw the same patient and the interesting concept that the condition was the result of hyperchondroplasia (as opposed to an achondroplasia) was presented. In 1902 Achard noted the marked attenuation of the extremities as well as the excessive length. Due to the spider-like appearance of the hands the condition was called arachnodactyly. Salle in 1912 first reported on the association of ocular and cardiac anomalies with the skeletal defects. In the first recorded autopsy Salle noted an exostosis of the sella turcica with an increased number of eosinophilic cells in the pituitary gland. The heart was dilated, the foramen ovale was patent and there were nodular lesions on the margins of the tricuspid and aortic valves.

The first case in American literature was reported in 1926 by Piper and Irvine-Jones.[2] At autopsy malformations of the lungs and heart were noted but the pituitary gland was normal.

Weve[1] in 1931 stressed the familial incidence of this relatively rare anomaly by reporting an adult male who had the characteristic ocular and skeletal changes. This patient had sixteen children, seven of whom had arachnodactyly. A father and three of his children, all afflicted with the same disorder, were also reported. Weve postulated that the syndrome was the result of a congenital mesodermal defect, since the tissues involved, i.e., skeletal muscle, heart, blood vessels and ligaments, are derived from mesoderm. However, no mention was made of the frequent pulmonary involvement which is endodermal in origin, nor the fact that the optic lens, an ectodermal structure, is involved in approximately 66 per cent of the reported cases. Futcher and Southworth[3] in 1938 emphasized the frequency of cardiac and pulmonary lesions in this syndrome.

In 1942 Rados[1] in a superb review of the subject, summarized the literature, the theories as to etiology and the clinical manifestations in 204 cases.

Baer, Taussig and Oppenheimer[4] reported two cases which presented aneurysm of the aorta with malformation of the aortic media. In 1947 Tobin, Bay and Humphreys[5] reported the pathologic findings of arachnodactyly in two cases and reviewed the autopsy findings in a total of fourteen cases reported in the literature. Six of fourteen cases demonstrated aortic lesions in which the histologic pattern could not be differentiated from Erdheim's[6] medionecrosis idiopathica cystica.

In 1949 Moehlig[7] reported on two cases of arachnodactyly in which hormonal assays were performed. Seventeen-ketosteroid excretion was found to be slightly elevated in a six year old boy who had previously been treated with methyl testosterone, and normal in a fourteen year old boy. He reported follicle stimulating hormone assays to be within normal limits in both patients. It is postulated by Moehlig[7] that this syndrome is the result of a primary inherited pituitary disturbance with a secondary mesodermal tissue defect.

CLINICAL MANIFESTATIONS

The clinical manifestations are conveniently divided into three groups:

1. *Musculo-skeletal.* The limbs are extremely long and slender. This characteristic is especially obvious in the metacarpals and phalanges and thus results in the descriptive appellation "spider fingers." The patient's span is greater than his height, and the lower segment is longer than the upper segment. These findings are exaggerated by the poor muscular development and the

* From the Department of Medicine, Section of Endocrinology and Metabolism, Ohio State University, Columbus, O.

paucity of subcutaneous tissue. The skull is long and narrow (dolichocephalic) and the supraorbital ridges are prominent so that the eyes appear to be deeply set. A high arched palate is usually seen. Due to the hypotonicity of the muscles and the laxity of the ligaments, there is hypermobility of the joints and many resultant deformities are seen, among which are funnel chest, pigeon chest, kyphosis, scoliosis and winged scapulas.

2. *Visceral. Cardiovascular:* In twelve of the fourteen cases which were autopsied cardiovascular lesions were found. The most common lesion is patent foramen ovale, but patent interauricular and interventricular septal defects, valvular lesions which cannot be differentiated from rheumatic heart disease, and aneurysms of the aorta have been reported. In fact, Tobin and his associates[5] state that in any case of dissecting aneurysm of unknown etiology arachnodactyly should be suspected.

Pulmonary anomalies are apparently frequent complications. In five of the fourteen autopsied cases reported in the literature pulmonary defects have been found. These consisted of variations in segmental lobular development and vestigial lobes with resultant increased susceptibility to pulmonary infections.

3. *The ocular anomalies* are found in approximately 50 per cent of the known cases. The most characteristic finding is subluxation of the lenses upward and outward with resulting tremulousness of the iris. Myopia is very common even in the absence of lens displacement. Microcornea, strabismus and nystagmus are also seen.

ETIOLOGY

No specific etiology is known. The high familial incidence of cases has been mentioned in the review of the literature and therefore the tendency would be to classify arachnodactyly as a genetic or inherited anomaly of development. Weve's[8] thesis that it is a result of genetic deficiency of mesodermal tissue is widely accepted. However, the ocular and pulmonary anomalies that are so frequently seen are not of mesodermal origin. It is unlikely that genetic influences are restricted to a single germ layer during embryonic development.

The role of the endocrines remain obscure. Pituitary involvement was reported in six of fourteen postmortem examinations of patients with this syndrome. In five of the six cases with pituitary abnormality eosinophilic hyperplasia was reported. A cyst of the pituitary was reported in the sixth patient. Hormone assays have been performed in the two cases reported by Moehlig[7] and the results must be considered within normal limits.

The generally accepted etiology is that of a genetic disorder which may or may not involve the endocrine system.

CASE REPORT

L. K., a thirty-five year old white male, was first seen at the University Hospital Surgical clinic for cellulitis of the left lower leg. Since the patient obviously manifested defective vision, he was referred to the Ophthalmologic Clinic where the subluxation of the lenses was noted and a diagnosis of Marfan's syndrome made. (Fig. 1.)

The patient was admitted to the medical service of University Hospital on December 6, 1949. The chief complaint was fatigue and weakness as long as he could remember. The fact that the patient was "different" was first recognized by his parents when he was five or six years of age. It was noted that the patient was taller and his arms and legs longer and thinner than those of his contemporaries. The patient was able to participate in most of the usual boyhood activities until the age of fifteen when he felt too weak to continue school. He was able to deliver newspapers until the age of nineteen when even this proved too fatiguing to continue. He has been unable to perform any remunerative work since that time. His average weight since attaining maturity has been 117 pounds with a maximum peak of 122 pounds in 1945.

In 1947 the patient first noted ankle edema and that his feet became cyanotic if they were dependent. He soon developed an ulceration of the left leg which was refractory to all attempted treatment.

The family history was pertinent in that no similar case was recognized in any sibling.

Physical examination revealed a tall, asthenic white male who was extremely slow and deliberate in all movements. The skull was dolichocephalic and the supraorbital ridges were moderately prominent. The patient's eyes were deeply set. There was bilateral displacement of the lenses upward and outward and the iris was tremulous. A high palatine arch was seen. The lungs were clear to percussion and auscultation and no abnormalities of the heart were noted.

The liver was palpable one finger below the costal margin. The genitalia were those of a mature adult male although the prostate was considered to be slightly smaller than normal. There were no pathologic reflexes. Inspection of the musculoskeletal system revealed very long slender extremities, with poorly developed musculature. The subcutaneous tissues were poorly developed and bony prominences were unusually obvious. The metacarpals, metatarsals and phalanges appeared particularly long. On admission there was increased pigmentation of both lower legs associated with pitting edema. There was a greyish ulcer about 4 cm. in diameter on the medial aspect of the left leg.

Measurements taken were as follows: height, 182 cm.; span, 192 cm.; symphyses to floor, 96 cm.; weight 118½ pounds (52.9 kg.).

The cellulitis and edema were treated with penicillin 300,000 units each day, priscoline® 50 mg. each day and elevation of the extremities. The patient responded slowly and continuously to this therapy.

Laboratory data: Red blood cells 4.93 with 11.7 gm. hemoglobin; hematocrit 46 per cent, white blood cells 9,900 with an essentially normal differential count. Bleeding and coagulation times were normal. Prothrombin was 58 per cent of normal. Bone marrow biopsy was normal. Serologic tests for syphilis were negative. Routine urine tests revealed no abnormalities. The patient was able to concentrate to a specific gravity of 1.033 and dilute to 1.003. The phenolsulfonphthalein excretion was 80 per cent. The total protein was 6.5 gm. with 4.6 gm. of albumin and 1.9 gm. of globulin per 100 cc. serum. The serum sodium was 335 mg. per cent, serum potassium 21.9 mg., serum chlorides 486 mg. per cent. The total cholesterol was 170 mg. per cent. The serum bilirubin was 0.3 units and the cephalin flocculation was negative. There was no bromsulfalein retention in forty-five minutes.

Gastric analysis revealed no free acid, but on stimulation with histamine free acid was obtained. A twelve-hour gastric secretion totaled 50 cc. An oral glucose tolerance test showed a fasting blood sugar level of 94 mg. per cent, one-half hour, 118 mg. per cent; one hour, 123 mg. per cent; 2 hours, 85 mg. per cent; three hours, 61 mg. per cent. Venous pressure was found to be 104 mm. of H_2O and circulation times were arm to lung six seconds and arm to tongue sixteen seconds. The electrocardiogram tracing was normal.

FIG. 1. L. K., aged thirty-five, demonstrates the characteristic appearance of a patient with Marfan's syndrome.

X-ray studies revealed a normal sella turcica. The cortices of the skull and long bones were thinner than normal. Excessive length of the metacarpals, metatarsals and phalanges was described. The x-ray of the chest revealed emphysema with decreased transverse diameter of the heart. Cholecystogram, upper gastrointestinal studies and barium enema were all considered to be within normal limits.

Since it was of particular interest to determine the potential role of the endocrine system in the production of this syndrome, the following additional studies were performed.

Pituitary: The sella turcica was not enlarged. The excretion of gonadotrophins in the urine was within normal range as determined by the mouse ovarian hyperemia technic. There was a normal response to the administration of 0.3 cc. of adrenalin subcutaneously in that the eosinophils in the peripheral blood fell from 272 per

cu. mm. to 111 per cu. mm. in four hours. This was indicative of a normal hypothalamic-pituitary-adrenal axis as postulated by Thorn and Forsham. There was a normal response to the insulin glucose tolerance test. Since there is no known, direct method of determining

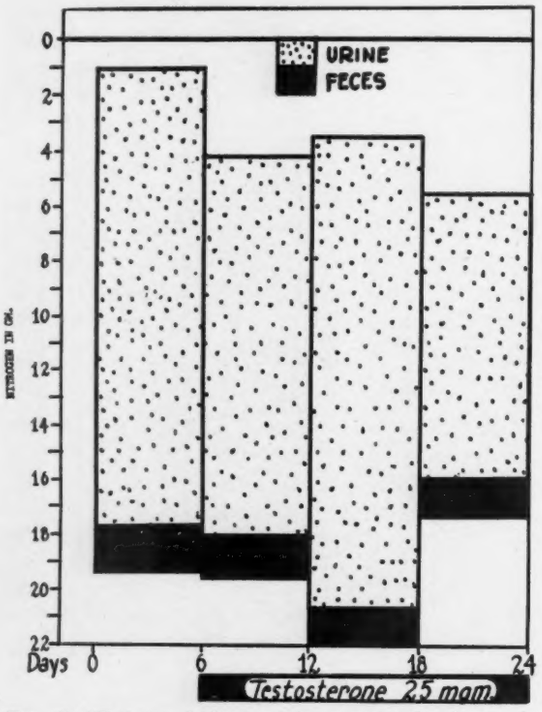

FIG. 2. Nitrogen balance; nitrogen intake for each period is recorded as the inferior border of each column. The fecal nitrogen excretion is recorded in solid black; urinary nitrogen excretion is recorded as the stippled area. The clear area between the top of the column and the "O" line represents the average amount of nitrogen retained daily during each period.

excessive amounts of growth hormone, the normal level of serum phosphorus is taken as indirect evidence that no abnormal growth hormone activity was present at the time this patient was seen.

Thyroid: The BMR was −14 per cent on three occasions and the serum cholesterol level was 170 mg. per cent. The serum protein-bound iodine was determined after cholecystography and the results were invalid. Unfortunately, a supply of I^{131} was not available at this time, therefore, tracer studies were not performed.

Parathyroids: The serum calcium, inorganic phosphorous, total protein and alkaline phosphatase were within normal limits.

Pancreas: The glucose tolerance and insulin glucose tolerance were considered to be within normal limits.

Adrenals: The 17-ketosteroid excretion was 16.0 mg. per twenty-four hours. Although the excretion of the 11-oxysteroids was not determined chemically, the normal fall in eosinophils after the injection of adrenalin is indicative of normal responsiveness of the adrenal cortex. The Robinson-Kepler-Power test of water excretion was normal and the serum electrolyte pattern was within normal limits.

Testes: Specimens of prostatic and testicular secretions were unobtainable by prostatic massage. In spite of the normal size and consistency of the testes, histologic study of a testicular biopsy revealed sparse tubular epithelium. Spermatogonium and a few spermatozoa were seen. A few small islands of Leydig cells were present in the interstitial tissues. The histologic diagnosis was testicular atrophy. Although the total 17-ketosteroid excretion was within normal limits, it is impossible to state that the testes secreted a normal amount of androgenic hormone.

In an effort to determine whether the tissues were capable of responding to stimulation by retaining nitrogen, metabolic balance studies were undertaken. The patient was placed on a weighed diet of approximately 2,770 calories made up of carbohydrate 295 gm., protein 122 gm. and fat 120 gm. with 1.2 gm. calcium and 2.0 gm. of phosphorus. Uneaten portions were weighed and subtracted from the intake and then aliquots of each meal were taken for analysis. Food, urine and feces were analyzed for nitrogen by the macro-Kjeldhal[9] method, for calcium[10] by the method of McCruden and for phosphorus[11] by the method of Subbarow and Fiske. Suitable daily aliquots were pooled and the study was divided into four periods of six days each. Daily determinations of creatinine[12] were made to verify the accuracy of urine collections. Seventeen-ketosteroid excretion was also determined daily using the method of Drekter, Pearson, Bartczak and McGavack.[13]

The patient was placed on the calculated diet for three days prior to starting the balance studies. Then after a six-day control period 25 mg. of testosterone propionate was given intramuscularly each day for three periods of six days each. As demonstrated by Kochakian[14] testosterone propionate is a potent stimulus to protoplasmic growth as defined by retention of nitrogen and phosphorus by the organism. If the patient's tissues were unable to respond to

this stimulus, there would be no increased retention of nitrogen and phosphorus and no increase in weight.

As can be seen from Figure 2, during the control period the patient retained nitrogen to the extent of 1 gm. per day. When testosterone propionate was administered, there was a definite increase in nitrogen retention to the range of 3.5 to 5.4 gm. of nitrogen per day. In the eighteen-day period that testosterone was administered, there was a total retention of 78.6 gm. of nitrogen. If this is multiplied by the accepted factor (32) to convert grams of nitrogen into fat-free protoplasm, the patient theoretically should have gained a total of 2,515 gm., or 5.6 pounds in the eighteen days. The patient actually gained 8 pounds, the difference being accounted for by sodium and water retention. (Fig. 3.)

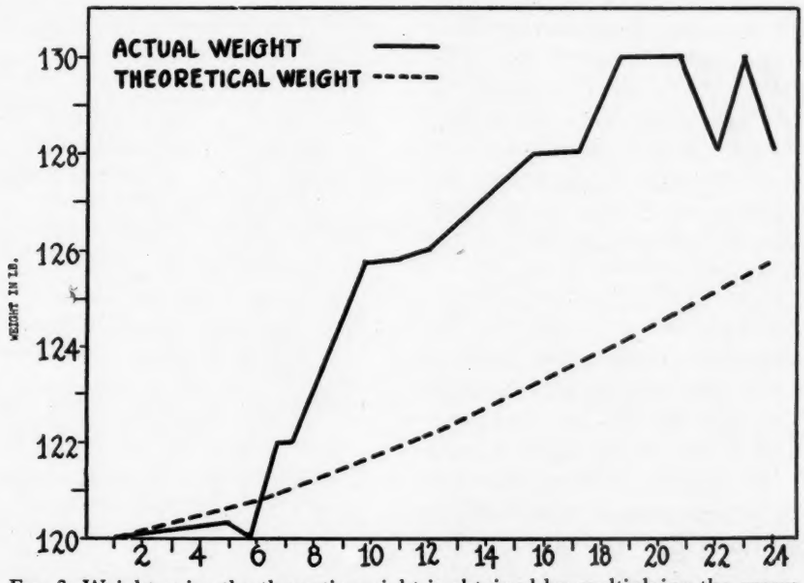

FIG. 3. Weight gain; the theoretic weight is obtained by multiplying the grams of nitrogen retained by the factor 32 which converts grams of nitrogen into grams of wet protoplasm.

That this gain in weight was the result of true growth, i.e., tissue anabolism, and not due to simple retention of salt and water, is confirmed by the phosphorus balance. (Fig. 4.) There were difficulties in the chemical analysis of calcium. The figures are not presented as they are not considered valid.

The creatinine coefficient is generally accepted to be an index of functioning muscle mass. It is derived by dividing the urinary excretion of creatinine in mg. per twenty-four-hour period by the weight in kg. Normal values for women are 14 to 22 while for men the accepted range is 20–26. In our patient the creatinine coefficient was found to be 22–26 when using the patients actual weight as the denominator in the ratio. This is a normal creatinine coefficient and, by inference, the patient had a normal amount of functioning muscle mass.

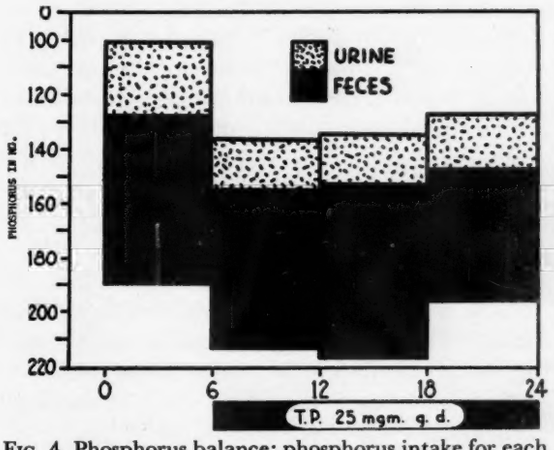

FIG. 4. Phosphorus balance; phosphorus intake for each period is recorded as the inferior border of each column. The fecal phosphorus excretion is in solid black. Urinary phosphorus is recorded in the stippled area. The clear area between the top of the column and the "O" line represents the average amount of phosphorus retained daily during each period.

Further evidence can be demonstrated from another point of view. The average creatinine excretion was 1.28 gm. per twenty-four hours. If the factor for the conversion of grams of urinary creatinine excreted in twenty-four hours

to muscle mass in kg. is accepted as 17.9, then 17.9 × 1.28 is equal to 22.9 kg. of muscle. The patient's weight was 53.6 kg. Therefore, muscle tissue made up 42.7 per cent of the patient's total body weight which is higher than the accepted average range of 30–35 per cent.[17]

The muscle mass would still be within normal range even if the so-called ideal weight were used instead of the actual weight. The Life Extension Institute tables[18] give 156 pounds or 70.8 kg. as the average weight of a thirty year old male whose height is approximately 6 feet. Using this figure as the basis of computing the proportion of muscle to total body weight, one arrives at the figure of 32.3 per cent which is still within the normal range.

CONCLUSION

Marfan's syndrome is characterized by skeletal, ocular and cardiovascular anomalies. Although these patients appear to be deficient in subcutaneous tissues and muscle, total muscle mass as derived from the creatinine coefficient has been shown to be within normal limits. It has also been demonstrated that the protoplasmic tissues are capable of responding to growth stimuli by storing nitrogen and phosphorus. No direct evidence could be obtained to implicate the endocrine system in an etiologic role. However, the possibility of some defect in intermediary metabolism involving formation, transport or deposition of fat cannot be ignored, since a gross deficiency of the fat stores in these patients is apparent. No attempt can be made to explain the apparent defects in the connective tissue supporting structures.

SUMMARY

1. A review of the literature concerning Marfan's syndrome is given and a case presenting the classical characteristics is discussed.

2. An attempt to find aberrations in the endocrine system was unsuccessful.

3. Nitrogen balance studies are presented which demonstrate the ability of tissue protoplasm to respond to growth stimuli.

Acknowledgements: I wish to express my appreciation for the valuable help given me by Mrs. Martha Lewis and Miss Madge Louise Myers of the Department of Nutrition and Mrs. Mary Brown Patton of the School of Home Economics. Dr. Irving Rothchild kindly performed the gonadotrophic assays which are reported. Thanks are also due the Schering Corporation for their generosity in supplying the testosterone propionate used in this study.

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Idiopathic Calcinosis Circumscripta*

GERALD I. PLITMAN, M.D. *and* BENJAMIN R. GENDEL, M.D.
Memphis, Tennessee

CALCINOSIS, the deposition of calcium masses in the skin and soft tissues, is an unusual and interesting condition. In its broadest sense the term calcinosis may refer to calcification of skin or soft tissues from any of several causes. These causes may be divided into four categories, namely, (1) mineral disturbances mediated by metabolic or endocrinologic abnormality; (2) calcification of hematomas or of tissues damaged by trauma or infection; (3) calcification associated with a vascular disturbance as in Raynaud's phenomenon or scleroderma and (4) calcification without obvious accompanying or preceding pathologic change.[1] In a stricter sense the term calcinosis refers to the latter two categories and the term idiopathic calcinosis to the lattermost category. It is to this group that the case herein presented belongs. The literature on this subject has been thoroughly reviewed at intervals in the past,[2–5] and will not be completely discussed here.

The etiology of this condition is unknown.[6,7] The varied theories of causation have included first, disorders of calcium metabolism and secondly, predisposition by tissue injury and death. In regard to the former it may be pointed out that in these cases there is no history of vitamin D intoxication; the urinary findings reveal no renal basis; and normal serum calcium and phosphorus values do not indicate parathyroid hyperactivity. With regard to the latter theory, in the usual case there is no known antecedent trauma and the studies of Bauer, Marble and Bennett[8] showed that, microscopically, early calcium deposition can be seen around normal-appearing fat tissue. Atkinson and Weber[2] reported the frequent coexistence of calcinosis and vasospastic lesions but Ghormley[3] found calcinosis to occur in only 2.5 per cent of a series of cases of vasospastic diseases studied at the Mayo Clinic.

Clinically the cases are divided into two groups: (1) calcinosis circumscripta in which the calcium deposits are of limited extent and confined to the skin and subcutaneous tissues, usually of the extremities; and (2) calcinosis universalis in which the calcification extensively involves the muscles and tendons as well. Although each type may occur at any time of life, calcinosis universalis is more common in childhood and youth while calcinosis circumscripta affects chiefly the older age groups. In each type the female sex is predominantly affected.[2,9] The degree of involvement may vary from only slight involvement, as in the case herein reported, to massive involvement as in the case of Moore and Clarke.[6] Symptoms vary according to site and degree of involvement and include the presence of nodules, pain in involved areas, limitation of motion of joints, general debility and sometimes the breakdown of superficial nodules to form sinuses which may discharge a cheesy, gritty material. Prognosis also depends upon the site and degree of involvement. The calcium deposits are, of course, readily demonstrable on x-ray.

Pathologically there is deposition of calcium in non-encapsulated clumps with surrounding fibrosis and infiltration of chronic inflammatory cells. Foreign body giant cells are frequently seen. There is, as a rule, no evidence of active tissue necrosis. Chemically, the deposits consist chiefly of calcium phosphate and calcium carbonate.

So varied have been the forms of attempted treatment that one gains the impression that no one method of therapy has been consistently successful. The relative rarity of the disease renders evaluation of a particular therapeutic regimen even more difficult. Moreover, the fact that spontaneous regressions have been reported makes any treatment even more difficult of

* From the General Medicine and Hematology Section of the Medical Service, Veterans Administration Medical Teaching Group, Kennedy Hospital, Memphis, Tenn. Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion and policy of the Veterans Administration.

FIG. 1. X-ray of the right arm showing multiple small calcific densities.

evaluation. Acid salts, iodides, ketogenic diet, x-ray therapy and parathyroidectomy have been tried with varying success.[4,10]

CASE REPORT

A fifty-six year old white man was admitted to the hospital with a complaint of hard deposits in the subcutaneous tissues of both arms of four years' duration. The appearance of these deposits was preceded in all instances by "burning and drawing" sensations but the masses themselves were asymptomatic except by virtue of their presence and progressive enlargement. In recent months the premonitory "burning and drawing" sensations had been noted in the right thigh posteriorly and in the right side of the face, but no hard masses had as yet appeared at those sites. There was no family history of this disorder and the patient gave no history of bone disease, renal stones or vitamin D ingestion. The past history was quite complex, the patient having had gonorrhea, syphilis, tuberculosis and influenza complicated by empyema many years before. He had had a number of operative procedures, chief among which were thoracotomy and construction of a perineal urethra following urinary extravasation due to urethral strictures.

Physical examination revealed a well developed, well nourished, white man who appeared neither acutely nor chronically ill. Temperature and pulse were normal. Blood pressure was 180/100. All teeth were absent. The old thoracotomy scar was noted in the fifth intercostal space in the right axilla. The lung fields were essentially clear to percussion and auscultation. The heart was not remarkable except for a rough apical systolic murmur. No abnormalities were noted in the abdomen except an old suprapubic cystostomy scar. The left testis was atrophic. An artificial urethra was present in the middle of the perineum. In the right arm there were several large, hard, irregular masses which seemed to lie in the subcutaneous tissues and did not move with contraction and relaxation of the muscles; a similar single mass was present in the left arm. Except for the surgical scars and subcutaneous masses, the skin was entirely normal. There was no evidence of peripheral vascular disease.

In the general laboratory work-up urinalysis and blood counts were within normal limits. Serologic test for syphilis was negative. Serum calcium level was 10.2 mg. per cent on each of two determinations; inorganic phosphorus was 3.6 and 3.0 mg. per cent on each of two occasions. Acid and alkaline phosphatases were 1.2 and 4.0 King-Armstrong units respectively. Urine concentration test revealed a maximum figure of 1.022; on a urea clearance test the patient showed 60 per cent of average normal clearance.

Electrocardiogram showed a right bundle-branch block. On chest x-ray the lungs were clear; there was, however, thickened pleura at both bases and an old resection of the right seventh rib. X-rays of the arms revealed numerous small, irregular, calcified densities varying in diameter from 0.1 to 0.3 cm. lying in the skin and subcutaneous tissues of both arms, (Fig. 1.) Similar calcifications were noted regional to the buttocks. X-rays of the long bones and spine showed no abnormalities except minimal osteoarthritic changes of the lumbar vertebrae.

During the hospital stay calcium excretion studies were accomplished by measuring the patient's twenty-four hour urinary calcium excretion while on a diet containing 137 mg. of calcium daily. The patient excreted 20, 40, 150 and 30 mg. of calcium on successive days during this time. During the first hospital week the patient had blood-streaked sputum. This, plus the history of tuberculosis, prompted the decision to have him bronchoscoped, including smears and cultures for acid-fast bacilli but all findings were normal.

Biopsies were taken from the following sites: (1) a calcified area in the right arm; histologically this showed dense broad bands of collagen in the corium, throughout which lay oval masses of amorphous calcified material surrounded by dense, hyalinized fibrous tissue. Several areas of chronic inflammation containing vacuolated foreign body giant cells were noted. (2) A "precursor" site in the right thigh at which symptoms of "burning and drawing" had been present but no calcification had been apparent; this was histologically normal. (3) A presumably "normal" site far removed from the other sites; histologically this was normal. All biopsy sites healed well.

Meanwhile, the patient had been receiving penile urethral dilatations by the Genito-Urinary Service with a view toward re-establishment of normal function. During the hospital course no specific therapy aimed at reducing the calcium masses was undertaken. Following completion of medical workup the patient was transferred to the Genito-Urinary Service where the urethrostomy was excised and function re-established in the penile urethra.

COMMENTS

The etiology and pathogenesis of idiopathic calcinosis is unknown. In view of the fact that this patient had certain premonitory symptoms of burning or drawing sensations prior to the deposition of calcium, it was thought that histologic study of such a "precursor site" might be informative. However, histologic study of the skin from such sites did not reveal any significant pathologic disorder. Some broadening of the collagen bands was noted but this did not differ from a similar variation in width of the collagen bands noted in sections of normal skin studied. In addition, no significant variations in the staining quality of the collagen could be detected. Study of our patient's skin removed from a normal area likewise showed no changes. No clue to the pathogenesis of this condition was afforded by a study of the many biopsies which this patient permitted. The etiology remains a mystery.

Lansbury[11] mentions briefly the controversy which has existed as to whether or not calcinosis actually represents a distinct entity, citing its frequent occurrence in association with Raynaud's phenomenon, scleroderma and dermatomyositis. He quotes Langmead[12] as having suggested that all these syndromes were manifestations of a single disease. The findings in the case discussed here suggest a separate existence of calcinosis; there were no skin changes, clinically or histologically, to suggest pre-existing disease such as scleroderma or dermatomyositis, and the proximal locations of the calcinotic deposits were inconsistent with their having been secondary to Raynaud's phenomenon.

SUMMARY

A case of calcinosis circumscripta in a middle-aged male is reported, and the pertinent features of the disease are reviewed briefly. The case herein described conformed to the rule in showing typical histologic changes and no demonstrable error of calcium metabolism.

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Book Review

Principles of Internal Medicine. Tinsley R. Harrison, M.D., editor-in-chief. 1,590 pages. Philadelphia, 1950. The Blakiston Company. *Price* $12.00.

This treatise is described on the flyleaf as "a new textbook of medicine . . . emphasizing the functional approach to specific disease treatment." The first two sections of the volume, "Cardinal manifestations of disease" and "Physiologic considerations," are indeed innovations in that they gather together information which is usually organized under discussions of the various specific disease processes in the classic textbook of internal medicine.

The excellence of certain chapters such as "Headache" and "Principal pathways of intermediary metabolism" justifies the new approach of the editors in reflecting the evolution of clinical medicine toward an emphasis on basic mechanisms instead of the older nosologic approach. Of the many admirable contributions the reviewer was particularly impressed by the sections on disorders of bone and of muscle, chemotherapy of infection and the collagen disorders.

Inevitably the first effort in an innovation produces results which in spots may be strained or inconsistent. The discussion of convulsions under the rubric of "Weakness" would seem rather artificial. The subject of therapy is inconsistently handled in the section on cardinal manifestations. For example, the chapter on "Pain in the chest" is concerned purely with mechanisms and differential diagnosis, and the reader is referred to the specific chapters in the last half of the volume for a discussion of treatment. On the other hand, in the chapter on "The painful back," definitive therapeutic measures are woven into the discussion.

The following comments are offered as suggestions for possible changes in a second edition: The chapter on inheritance of human disease is an excellent addition but could be made more useful if the description of genetic mechanisms were applied specifically to recent knowledge concerning such diseases as gout and thalassemia. In Chapter 18 values for the A/G ratio are still listed as being pertinent. The discussion of the Rh factors is quite incomplete. Mention should be made of the treponemia-immobilization test in the diagnosis of syphilis and of the role of the Coxsackie viruses in human disease. Measurements are inconsistently rendered in either the apothecaries' or the metric system.

Over-all, the editors are to be commended for having produced a highly readable, intelligently written volume which will be useful to medical student and internist alike. It is hoped that in succeeding editions the editors will continue to develop their original approach to the task of encompassing internal medicine within the confines of a single volume.

C.D.G.

Prelude to asthma?

not necessarily...

Tedral, taken at first sign of attack, often forestalls severe symptoms.

in 15 minutes...Tedral brings symptomatic relief with a definite increase in vital capacity. Breathing becomes easier as Tedral relaxes smooth muscle, reduces tissue edema, provides mild sedation.

for 4 full hours...Tedral maintains more normal respiration for a sustained period—not just a momentary pause in the attack.

Prompt and prolonged relief with Tedral can be initiated any time, day or night, whenever needed without fear of incapacitating side effects.

Tedral provides:

theophylline	2 gr.
ephedrine	3/8 gr.
phenobarbital	1/8 gr.

in boxes of 24, 120 and 1000 tablets

Tedral

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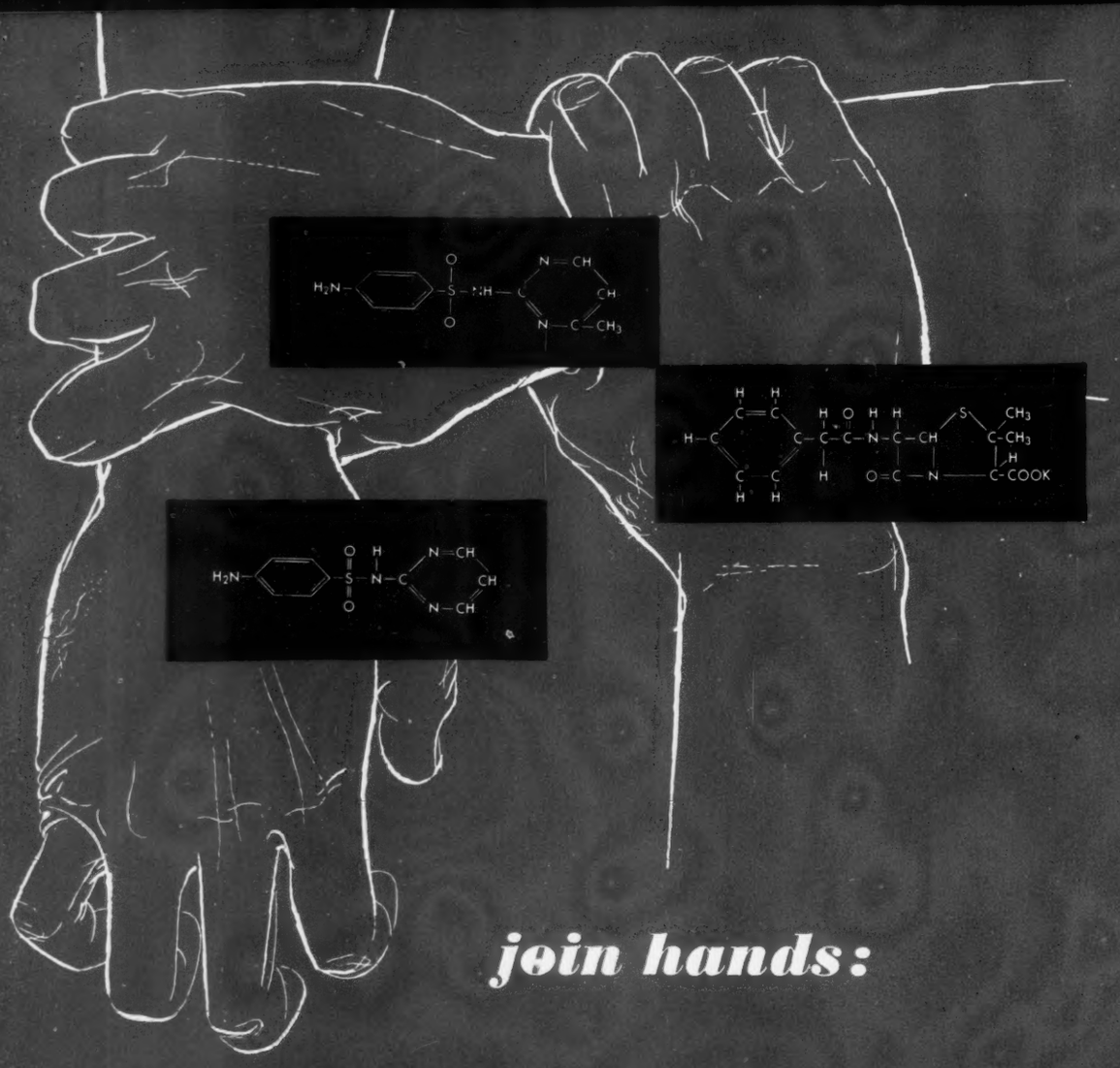

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In vitro and in vivo studies clearly demonstrate that in combating a wide range of organisms, penicillin, the *antibiotic* of greatest use, and the *sulfas* "join hands". To widen both antibacterial spectrum *and* effectiveness,

simply Rx **Biosulfa** *

Each tablet contains:

Penicillin G Potassium, Crystalline	100,000 units
Sulfadiazine	0.25 Gm.
Sulfamerazine	0.25 Gm.
Calcium Carbonate	4 grs.

Available in bottles of 50 tablets.

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to the

male climacteric patient

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ORETON®

(Testosterone Propionate U.S.P.)

ORETON multiple-dose vials of 10 cc., in oil for intramuscular injection — available in 25, 50 or 100 mg. per cc.

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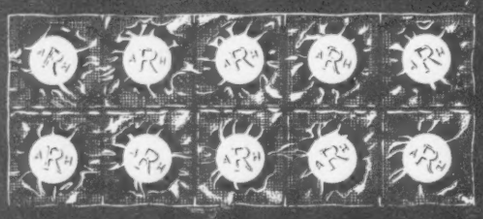
the man who



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Only by thwarting, somehow, the strong natural forces that normally repair lesions of gastric and duodenal mucosa, has the patient developed ulcer. Only by enlisting and implementing those forces effectively can ulcer be corrected. As an efficient antacid and demulcent, Robalate helps accomplish this with *no acid rebound, no change in acid-base balance,* and *no toxicity*. Robalate provides prompt and prolonged reduction of acidity, a rapid relief from pain. It covers the ulcer and the mucosa with a soft protective coating that favors restoration of normal function and healing. By *helping the ulcer help itself,* Robalate provides a smooth therapeutic course for the ulcer patient.

A. H. ROBINS COMPANY, INC.
Richmond 20, Virginia

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(Dihydroxy aluminum aminoacetate, N.N.R., 0.5 Gm. per tablet)

Robins

CHEMISTRY OF THE AMERICAN MEDICAL ASSOCIATION

Terramycin appears to be a drug of choice in "angular conjunctivitis, micrococcal blepharitis, marginal blepharitis, pneumococcal conjunctivitis, chronic dacryocystitis, serpent ulcer of the cornea, infection by Neisseria catarrhalis and acute purulent inflammation."

Mitsui, Y., et al.: Antibiotics and Chemotherapy (In Press).

"Trachoma and inclusion blennorrhea respond well to treatment with terramycin."

Mitsui, Y., and Tanaka, C.: Antibiotics and Chemotherapy 1:*146 (May) 1951.*

available: Crystalline Terramycin Hydrochloride Ophthalmic Ointment, 1 mg. per Gm. ointment, ⅛ oz. tube;

Crystalline Terramycin Hydrochloride Ophthalmic Solution, 25 mg. in 5 cc. dropper-vials.

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Iodine organically combined with thiouracil

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